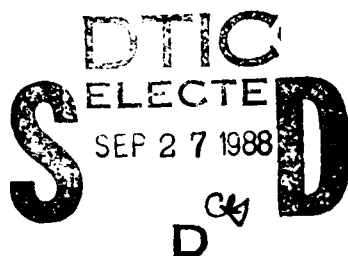


AD-A199 368



Technical Report 1060  
June 1988

# Marine Microcosm Experiments on Effects of Copper and Tributyltin-Based Antifouling Paint Leachates

R. Scott Henderson

Approved for public release; distribution is unlimited.

88 9 26 08 6

4

# **NAVAL OCEAN SYSTEMS CENTER**

## **San Diego, California 92152-5000**

---

**E. G. SCHWEIZER, CAPT, USN**  
**Commander**

**R. M. HILLYER**  
**Technical Director**

### **ADMINISTRATIVE INFORMATION**

The work described herein was performed by the Marine Environment Branch, Code 522, Naval Ocean Systems Center, for the Energy Research and Development Office, David W. Taylor Naval Ship Research and Development Center (Code 2759); the Office of the Chief of Naval Research (Code 123); and the Naval Facilities Engineering Command (Code 032).

Released by  
P. F. Seligman, Head  
Marine Environment Branch

Under authority of  
S. Yamamoto, Head  
Environmental Sciences  
Division

### **ACKNOWLEDGMENTS**

The author gratefully acknowledges the contributions of personnel who accomplished maintenance and operation tasks, chemical analyses, biological sorting and identification, and field collection work. At the Naval Ocean Systems Center (NAVOCEANSYSCEN) Hawaii Laboratory these persons included W. Cooke, R. Fransham, J. Groves, J. Grovhoug, and D. Pawloski. Contributors at NAVOCEANSYSCEN, San Diego, were C. Dooley, V. Homer, P. Kenis, S. Lieberman, P. Seligman, P. Stang, and A. Valkirs. T. Walsh and W. Young of Analytical Services, Honolulu, performed nutrient analyses.

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

## REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED			1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION/AVAILABILITY OF REPORT  Approved for public release; distribution is unlimited.	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE				
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NOSC TR 1060			5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION Naval Ocean Systems Center		6b. OFFICE SYMBOL (if applicable) Code 522	7a. NAME OF MONITORING ORGANIZATION	
6c. ADDRESS (City, State and ZIP Code)  San Diego, California 92152-5000			7b. ADDRESS (City, State and ZIP Code)	
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Energy R&D Office David W. Taylor Naval Ship R&D Center Naval Facilities Engineering Command		8b. OFFICE SYMBOL (if applicable) Code 2759 Code 032	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State and ZIP Code)  Annapolis, MD 21402-1198 Alexandria, VA 22332			10. SOURCE OF FUNDING NUMBERS	
			PROGRAM ELEMENT NO 63724N 63721N	PROJECT NO. Z0829 Y0817
			TASK NO. ME38 ME35	AGENCY ACCESSION NO. DN888 749 DN288 604
11. TITLE (include Security Classification)  MARINE MICROCOSM EXPERIMENTS ON EFFECTS OF COPPER AND TRIBUTYL TIN-BASED ANTIFOULING PAINT LEACHATES				
12. PERSONAL AUTHOR(S)  R. Scott Henderson				
13a. TYPE OF REPORT Final		13b. TIME COVERED FROM Oct 1980 TO Jun 1983		14. DATE OF REPORT (Year, Month, Day) Jun 1988
15. PAGE COUNT 52				
16. SUPPLEMENTARY NOTATION				
17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)	
FIELD	GROUP	SUB-GROUP		
			antifouling paint , organotin paint leachates , marine microcosms	
			benthic organisms, harbor pollutants tributyltin .	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)  A broad spectrum of marine plants and animals were excluded from flowthrough seawater microcosms by chronic exposures (2 to 4 months) to 0.5 to 1.8 µg/L-TBTO (tributyltin) derived from antifouling paints. Rapid recovery of leachate-treated microcosms demonstrated that residual organotins were depurated quickly from sediments and/or had low toxicity. Community metabolic studies showed that the net impact on organic productivity of bottom-living harbor organisms would probably be minimal at sub-µg/L TBTO concentrations. Gobiid fish and sea cucumbers experienced significant uptake of organotin-Sn over 2- to 3-month exposures to 0.8 µg organotin-Sn/L. Sediments took up organotin-Sn at a nearly constant rate during a 3-month exposure to 0.8 µg organotin-Sn/L, and rapidly released about 50 percent of absorbed tin in a 3-month depuration period. Two species of coral were found to be very sensitive indicators of total organotin concentrations. Toxic responses of corals indicated that, on a molar basis, TBTO-Sn was at least 10 times more toxic than copper.				
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS			21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL R. Scott Henderson			22b. TELEPHONE (include Area Code) (808) 257-1110	22c. OFFICE SYMBOL Code 522

DD FORM 1473, 84 JAN

83 APR EDITION MAY BE USED UNTIL EXHAUSTED  
ALL OTHER EDITIONS ARE OBSOLETEUNCLASSIFIED  
SECURITY CLASSIFICATION OF THIS PAGE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

8a. NAME OF FUNDING/SPONSORING ORGANIZATION (Continued)

Office of Chief of Naval Research (Code 123)

## SUMMARY

A broad spectrum of marine plants and animals were excluded from seawater microcosms by chronic exposures (of 2 to 3 months) to 0.5 to 1.8  $\mu\text{g/L}$  (ppb) of tributyltin oxide (TBTO) derived from antifouling paints. Microcosms are defined here as complex communities of organisms maintained in flowthrough tanks under natural conditions. A sampling of obvious effects on representative species showed that mortality rates were generally over 80 percent for invertebrate animals exposed to TBTO concentrations of 0.5  $\mu\text{g/L}$  or greater (table S-1).

**Table S-1.** Responses of selected microcosm organisms to paint leachates.

Animal	Paint Type and Leachate Concentration		
	F121 (5.6-ppb copper)	SPC-4 (1.7-ppb organotin, 1.3-ppb copper)	OMP-253 (0.5-1.8-ppb TBTO)
Sea Anemones	Normal	100% mortality in 23-42 days	1.8 ppb 90% mortality in 7 days
Sea Hares	Normal	At 92 days only 5 adults survived; all larvae/juveniles killed	1.8 ppb 100% mortality in 7 days 0.5 ppb 80% mortality in 28 days
Sea Cucumbers	Normal	At 92 days 1 dead, 2 with lesions, all 6 lost weight	At 2 days all stopped feeding, most developed lesions
Corals	5% mortality in 28 days	90% mortality in 21 days	0.5 ppb 95% mortality in 23 days

In general, effects of leachates were found to be greatest on animals in larval life stage and those lowest in evolutionary hierarchy (e.g., corals, anemones, echinoderms, and molluscs). Lesser effects were observed with adults and higher animals (e.g., polychaetes, crustaceans, and fish). Effects of organotin leachates on algae were much less than noted on invertebrates.

Leachate-exposed sediments accumulated moderate levels of organotin at constant rates and depurated 50 percent of that material within 3 months after termination of exposure. Toxicity of organotin-loaded sediments to bottom animals appears to be minimal as evidenced by rapid recolonization of sediments previously exposed to leachates for long periods.

Gobiid fish accumulated tissue concentrations of solvent-extractable tin 1,300 to 2,900 times higher than tin concentrations in their treatment water. Although the fish showed no obvious negative effects, evaluation of potential higher-level food-chain effects will be dependent on future identification of actual organotin species present in animal tissues.

Results from the microcosm studies strongly suggest that tributyltin (TBT) release rates from organotin antifouling coatings must be minimized to substantially reduce environmental inputs of TBT leachates to ensure that TBT concentrations in harbors and estuaries do not exceed 0.5  $\mu\text{g/L}$  TBT to avoid risk of significant damage to sensitive faunal groups. Future chronic studies will better define lower safe limits of TBT.

# CONTENTS

SUMMARY .....	i
ILLUSTRATIONS .....	iv
TABLES .....	v
SECTION 1. EXPERIMENTAL DESIGN AND WATER CHEMISTRY OF ANTIFOULING LEACHATE MICROCOSM EXPERIMENTS .....	
	1
INTRODUCTION .....	1
EXPERIMENTAL DESIGN AND PROCEDURES .....	2
Facility Description .....	2
Experimental Design .....	3
METHODS .....	7
F121/SPC-4 Comparison Experiment .....	7
OMP-253 Experiment .....	8
RESULTS AND DISCUSSION .....	8
F121/SPC-4 Experiment .....	8
OMP-253 Experiment .....	9
SECTION 2. POPULATION STRUCTURES OF BENTHIC MICROCOSMS EXPOSED TO ANTIFOULING LEACHATES .....	
	14
INTRODUCTION .....	14
METHODS .....	14
F121/SPC-4 Experiment .....	14
OMP-253 Experiment .....	15
RESULTS AND DISCUSSION .....	15
F121/SPC-4 Experiment .....	15
OMP-253 Experiment .....	18
General Discussion .....	21
SECTION 3. METABOLIC RESPONSES OF BENTHIC MICROCOSMS EXPOSED TO ANTIFOULING LEACHATES .....	
	23
INTRODUCTION .....	23
METHODS .....	23
F121/SPC-4 Experiment .....	23
OMP-253 Experiment .....	24

## CONTENTS (Continued)

RESULTS AND DISCUSSION .....	24
F121/SPC-4 Experiment .....	24
OMP-253 Experiment .....	26
General Discussion .....	28
SECTION 4. ORGANOTIN BURDENS OF MARINE ORGANISMS AND SEDIMENTS EXPOSED TO SPC-4 ANTIFOULING LEACHATES .....	30
INTRODUCTION .....	30
METHODS .....	30
RESULTS AND DISCUSSION .....	31
SECTION 5. EFFECTS OF ANTIFOULING LEACHATES ON TWO SPECIES OF CORAL .....	35
INTRODUCTION .....	35
METHODS .....	35
RESULTS AND DISCUSSION .....	36
REFERENCES .....	39
ABBREVIATIONS AND SYMBOLS .....	41



A-1

✓

## ILLUSTRATIONS

1. Plumbing schematic (upper) and plan-view (lower) of one microcosm tank as configured for the antifouling paint leachate experiments . . . .	3
2. Experimental phases of the F121/SPC-4 comparison experiment and the OMP-253 experiment . . . . .	5
3. Schematic of microcosm treatments used in the OMP-253 leachate effects experiment . . . . .	6
4. Time-series plots of mean dissolved copper concentration of tank water and mean calculated copper leach rates for antifouling panels in treated microcosms . . . . .	9
5. Organisms typical of soft-bottom microcosm communities . . . . .	15
6. Infauna abundance in various treatment tanks of the F121/SPC-4 leachate experiment at ends of treatment and recovery phases . . . . .	17
7. Infauna abundance in various treatment tanks of OMP-253 leachate experiment at ends of recovery phase I, treatment phase II, and recovery phase III . . . . .	19
8. Peak O <sub>2</sub> production for F121 and SPC-4 leachate treatment microcosms . . . . .	25
9. Peak O <sub>2</sub> consumption for F121 and SPC-4 leachate treatment microcosms . . . . .	25
10. Integrated diurnal O <sub>2</sub> production and consumption for HiRes and LoRes OMP-253 leachate treatment microcosms . . . . .	27
11. Integrated diurnal O <sub>2</sub> production and consumption for HiOrg OMP-253 leachate treatment microcosms . . . . .	27
12. Uptake and depuration of organotin-Sn in microcosm sediments exposed to a mean concentration of 0.8 µg/L of organotin-Sn SPC-4 leachate . . . . .	34
13. Survival of two coral species ( <i>Montipora verrucosa</i> and <i>Pocillopora damicornis</i> ) exposed to leachates from 0.1 m <sup>2</sup> of F121 (copper toxin) or SPC-4 (organotin and copper toxins) paint . . . . .	37
14. Survival of two coral species ( <i>Montipora verrucosa</i> and <i>Pocillopora damicornis</i> ) exposed to various treatments of OMP-253 paint leachates from a 0.1 m <sup>2</sup> panel . . . . .	38



## TABLES

1. Concentrations of TBTO and dibutyltin chloride (DBTC1) for water of one tank from each 0.5 m <sup>2</sup> OMP-253 leachate treatment and one control tank .....	10
2. Organotin concentrations in 0.1 m <sup>2</sup> OMP-253 treatment tanks and a control tank .....	11
3. Chlorophyll $\alpha$ concentrations for Contr and HiOrg microcosms and added culture .....	12
4. Particulate and dissolved organic carbon and chlorophyll $\alpha$ content of Contr and HiOrg microcosm water .....	13
5. Macroalgae observed in OMP-253 leachate microcosms during treatment phase II .....	20
6. Tin content of a gobiid fish ( <i>Bathygobius fuscus</i> ) maintained in microcosms for the F121/SPC-4 leachate effects experiment .....	32
7. Concentration of total organotin and organotin species in microcosm sediments exposed to SPC-4 leachates .....	33

## SECTION 1.

# EXPERIMENTAL DESIGN AND WATER CHEMISTRY OF ANTIFOULING LEACHATE MICROCOSM EXPERIMENTS

## INTRODUCTION

The experiments described herein are part of a major program designed to evaluate possible environmental effects of Navy Fleet use of organotin antifouling paints. These experiments were performed using microcosms—defined here as flowthrough outdoor aquaria housing natural complex communities of marine organisms. The microcosms are used as small-scale functional and structural models of natural harbor ecosystems.

The application of intermediate- to large-sized microcosms to environmental research has grown immensely in the last two decades (Giesy, 1980; Grice & Reeve, 1982). Increasing numbers of researchers and environmental managers note that data relating to pollutant effects on intact, near-natural communities yield much better approximations of real-world conditions than do data obtained from traditional bioassay experiments using single species of organisms in restricted, abnormal settings.

Maintained in nearly identical conditions, replicate microcosms provide a group of miniature ecosystems that can be subjected to controlled environmental stresses. By comparing the metabolism and structure of stressed microcosms to those of control microcosms, the aggregate responses of complex, functional communities to pollutants, such as oil, heavy metals, waste heat, sewage, and pesticides, can be determined. Microcosm investigations also provide a useful bridge between noninteractive tests (e.g., bioassays) and complex, interactive field experiments and monitoring programs.

Whereas bioassay tests continue to be useful tools in the preliminary screening of potentially hazardous materials, microcosm experiments provide empirical facts about the fates and effects of chemicals that result in the fine-tuning of hazard evaluations. As recognized by the Environmental Protection Agency (Draggan & Reisa, 1980), microcosm test data will play an increasing role in the evaluation of ecological effects, gradually replacing tests that do not adequately simulate the character of natural environments.

The microcosm facility at the Naval Ocean Systems Center (NOSC) Hawaii Laboratory has been used for several years to examine the effects on benthos of common harbor pollutants, such as sewage nutrients, heavy metals, excess heat, fresh water, and hydrocarbons. In the last 2 years, research efforts have focused on changes in mud-bottom communities caused by copper and organotin leachates from antifouling paints. These studies have been initiated because the Navy, which uses primarily copper toxicant antifouling paints on ship hulls, is now considering wide-scale conversion to recently developed copolymer paints with organotin toxicants.

A major advantage of copolymer paints is that release of toxicants is slow and uniform because they are chemically bound to compounds in the paint matrix. In conventional copper paints, toxicants are suspended in the paint matrix. Thus, toxicants in noncopolymer paints leach at higher and more variable rates that are generally dependent on the character of the oxide and salt coatings that form over the paint surfaces. Because of slower and more controlled toxin leach rates, organotin copolymer paints exhibit a considerably longer effective antifouling life than copper paints. Organotin copolymers also have more effective protective action against animal foulers. Additionally, they do not enter into electrolytic reactions with metal hulls, and most are self-polishing as they are designed to ablate at a continuous rate.

Presently relatively little is known about the fates and effects of organotin compounds in marine environments. Although it is generally recognized that the most commonly used toxic compounds (trialkyltins) decrease in toxicity as they lose alkyl groups, there is little agreement on what processes are most important in degradation or at what rates breakdown and detoxification occur.

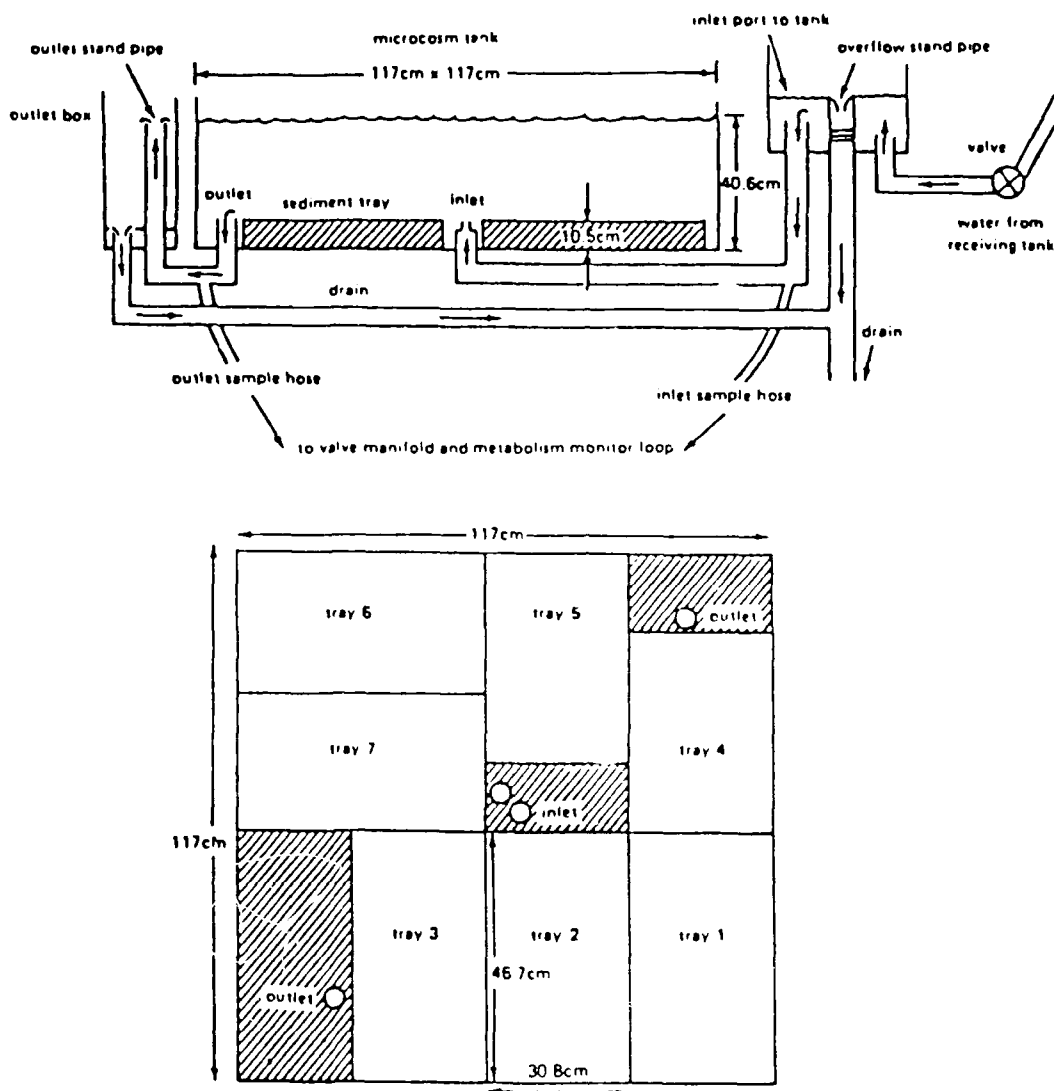
The first of the present experiments, performed from October 1980 to November 1981, compared the "fresh" (nondegraded) toxicity of an organotin copolymer paint leachate to that of a copper-based antifouling paint widely used by the Navy. In the second experiment, performed from December 1981 to February 1983, toxicities were determined under differing environmental conditions for various aged leachates derived from a Navy-developed organotin copolymer paint.

## **EXPERIMENTAL DESIGN AND PROCEDURES**

### **Facility Description**

These experiments were conducted at the NOSC Ulupau Marine Microcosm Facility located on Mokapu Peninsula, Oahu, Hawaii. Unfiltered seawater was pumped from an open coast reef flat to a large-volume receiving tank and thence to flow control boxes that feed water to 12 fiberglass microcosm tanks (figure 1). Each tank is 528 L in volume and measures 117 by 117 by 40 cm deep. Detailed descriptions of the plumbing, sampling, and monitoring systems associated with the microcosms are provided in Henderson and Smith (1980).

All tanks were situated in open sunlight, but were shaded with a layer of fiberglass window screen to reduce light levels in the tanks by 36 percent and to contain active organisms such as gobiid fish in the tanks. Seawater flow rates through the tanks were maintained at about 7 L/min to produce a water residence time of about 1 hour (for tank water volumes of about 465 L with 62 L of sediment). This flushing rate is sufficient to minimize external heating and cooling effects and harmful buildup of metabolic products such as carbon dioxide and ammonia in the microcosms. Supply water temperatures vary seasonally from 22° to 28 °C and salinities vary from 34 to 36 ppt.



**Figure 1.** Plumbing schematic (upper) and plan-view (lower) of one microcosm tank as configured for the antifouling paint leachate experiments.

## Experimental Design

**F121/SPC-4 Comparison Experiment.** Formula 121/63 copper toxicant paint and self-polishing copolymer formula 4 (International Paint Corp. SPC-4) organotin toxicant paint were used as sources of leachates for treatments in this experiment. F121/63 paint contains 70-percent cuprous oxide and is presently the most common antifouling paint used by the Navy. Wet weight composition of SPC-4 is 11.7-percent bis(tri-n-butyltin) oxide (TBTO) and 17-percent cuprous thiocyanate in a polymeric matrix. SPC-4 was selected as a typical organometallic copolymer paint that was under U.S. Navy testing at the time this test was run. Subsequently, the production of SPC-4 formulation was discontinued in favor of lower release rate formulations. SPC-4 paint was applied by a paint roller in two coats onto plexiglass panels painted with a single coat of Navy F150 epoxy undercoat.

The purpose of this experiment was to examine and compare the effects of leachates of the two paint types on marine communities typical of harbor environs. Painted panel area per tank treatment was selected to approximate conditions in Southeast Loch, Pearl Harbor, a typical low-circulation harbor area containing Navy craft of all sizes. Using data on the average wetted (antifouling painted) hull surface area present and water flushing rate in Southeast Loch, 0.5 m<sup>2</sup> of painted panel area in each tank with a 7 L/min flow was calculated to be appropriate to scale leachate input in the microcosms to that in a harbor with low flushing rate.

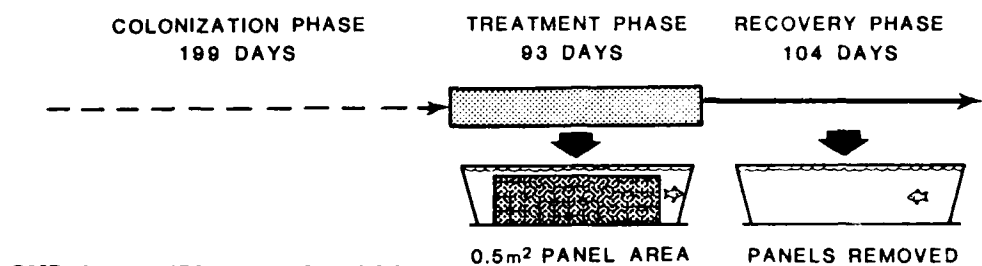
Antifouling paint panels were leached in flowthrough tanks for 2 months before experimental use so toxicant leach rates would decline to levels more representative of a multiship source. Panels coated with a single coat of F150 epoxy were used in control tanks to produce shading on walls equivalent to that created by panels in treatment tanks. During the treatment phase, SPC-4 panels were removed from the tanks twice weekly and were gently sponged off to simulate the ablative "self-polishing" action of boat motion on the SPC-4 paint surfaces. Out-facing sides of control panels were brushed twice weekly to remove fouling growth.

In preparation for the experiment, four boxes of dried calcareous mud and three boxes of a 1:1 mix by volume of dried calcareous mud and quarried calcareous rubble were added to each of nine microcosm tanks (figure 1 shows tray dimensions and orientations). Seawater flow was then provided to the tanks for 6-1/2 months to allow natural colonization and stabilization of organism populations before beginning the treatment phase of the experiment. Some field-collected organisms were introduced to the microcosms and details of those additions are given in section 2.

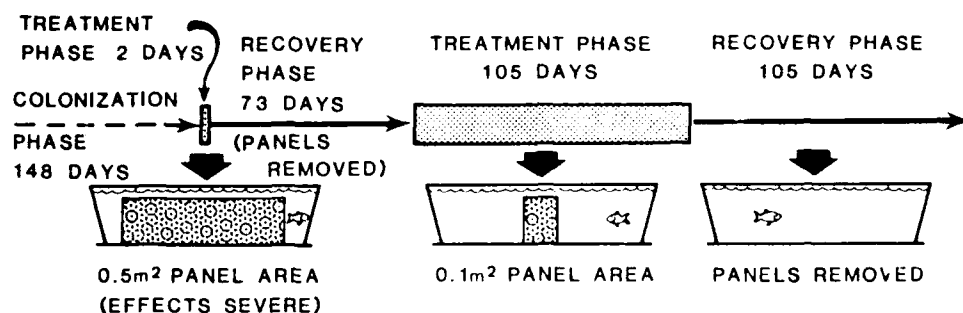
At the beginning of the 93-day treatment phase (figure 2), 0.5 m<sup>2</sup> of control or antifouling panels were added to each microcosm tank. Three microcosms were used for control and F121/63 and SPC-4 treatments. During the treatment phase, water samples for copper analysis were collected from control and F121/63 microcosms eight times, and samples for organotin analysis were collected from control and SPC-4 microcosms seven times. Additionally, two sets of water samples were taken from SPC-4 tanks for copper analysis. After 93 days of leachate exposure, the panels were removed from the tanks and the microcosms were allowed a 104-day recovery phase. No water samples were taken during the recovery phase.

OMP-253 Experiment. OMP-253, an organometallic copolymer paint containing 22.6-percent TBTO by weight, was used as a leachate source in this experiment. This paint has undergone development and testing at the David W. Taylor Naval Ship Research and Development Center and has been considered for use on Navy ships. A single coat of OMP-253 was sprayed onto plexiglass panels. In the first treatment phase (figure 2), a painted panel area of 0.5 m<sup>2</sup> was used in each OMP-253 treatment so paint areas would be identical to those used in the F121/SPC-4 experiment. However, because of the unexpected high leach rate of the paint and severity of effects on biota resulting from the 0.5 m<sup>2</sup> OMP-253 treatments, exposures were terminated after only 2 days. Follow-on exposures used panel areas of only 0.1 m<sup>2</sup> per tank. A water sample for organotin content analysis was collected from one tank of each treatment during this first treatment phase.

# SPC/F121 EXPERIMENT 1981



# OMP-253 EXPERIMENT 1982

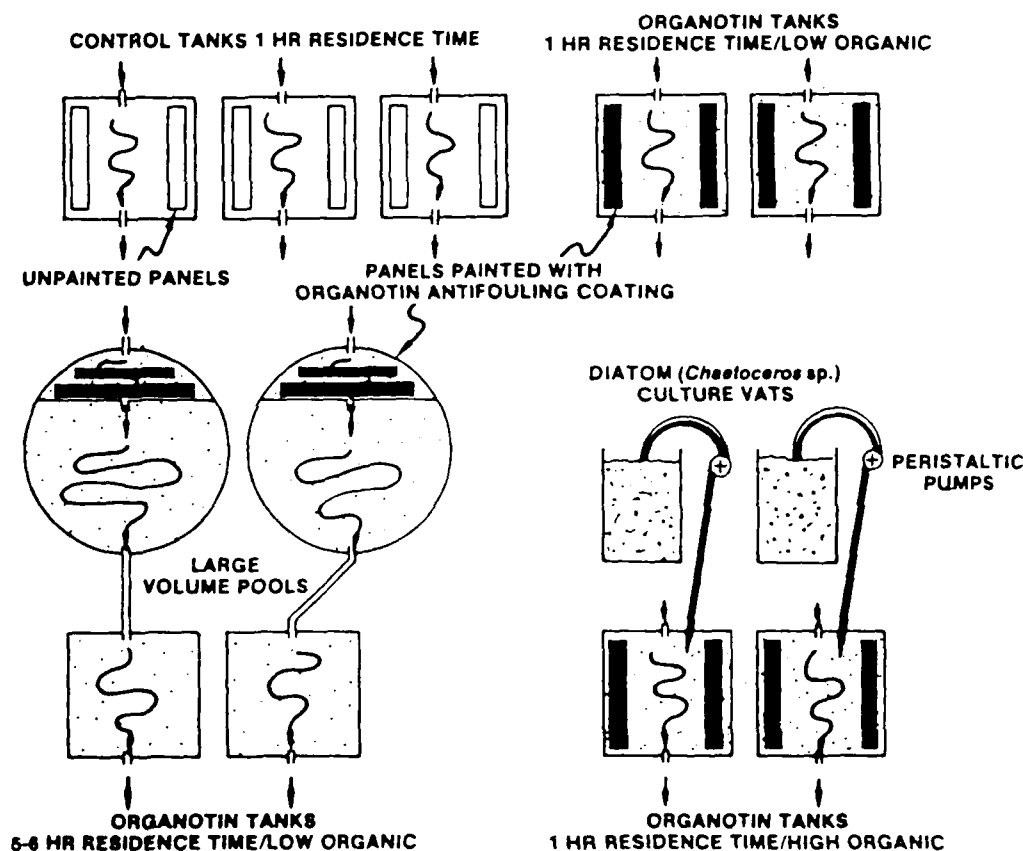


**Figure 2.** Experimental phases of the F121/SPC-4 comparison experiment and the OMP-253 experiment.

Three different treatments of OMP-253 leachate were selected to examine the effects of extended exposure time and the presence of organic material on the concentration of organotins (figure 3). Abbreviated designators in brackets in the following treatment descriptions will be used as standard descriptors for those treatments throughout this report.

One pair of microcosm tanks (LoRes) containing OMP-253 panels received low-organic ambient seawater at a flow rate of 7 L/min, yielding a low average residence time of water (1 hour) in those tanks. Upstream of each of two other microcosm tanks was a vinyl-lined circular pool (HiRes-Pool) of 2,400-L volume that received water at 7 L/min and contained OMP-253 panels. The average residence of water in each pool was 5.7 hours before the water entered a microcosm tank (HiRes). Thus, leachates entering the microcosms from the pools had been allowed nearly six times the exposure to ambient water as had leachates in low-residence-time treatments. Hypothetically, dissolved organotin concentrations and toxic effects would be decreased in these treatments because of the longer time allowed for adsorption of organotins before the leachates came into contact with the microcosm communities.

A third pair of microcosm tanks (HiOrg) containing OMP-253 panels were configured for short-residence-time flushing (similar to the LoRes tanks), but were supplied with continuous input of concentrated phytoplankton culture to increase particulate organic levels of the microcosm waters several times above ambient water to levels typical of high-nutrient, estuarine harbors. In theory, toxicity of organotins would have been reduced in these tanks because of adsorption and uptake of leachate compounds by the phytoplankton.



**Figure 3.** Schematic of microcosm treatments used in the OMP-253 leachate effects experiment. One control microcosm was converted to a 1-hour residence time/low organic treatment for treatment phase II and recovery phase II.

Plankton material added to the HiOrg tanks was cultured in batches by additions of culture inoculum and nutrient solution (standard F/2) to sterilized seawater. Climax cultures used for additions consisted of varying proportions of *Chaetoceros* sp. (a diatom), *Tetraselmas chuii* (a chlorophyte), and chained blue-green algae, species that are common components of harbor phytoplankton populations. Cell counts of added culture were in the range of 1 to 10 times  $10^6$ /ml, and that material was metered into the microcosms at a rate of about 0.1 L/min. Resultant dilution of culture water in the microcosms was about 70 fold.

Three low-residence-time microcosm tanks were used as controls (Contr-Tanks), which received no OMP-253 leachates. Panels in those tanks were equivalent in area to those placed in OMP-253 tanks but were painted only with Navy F150 epoxy paint.

After the 2-day  $0.5 \text{ m}^2$  panel exposure and a recovery period of 74 days, a panel of  $0.1 \text{ m}^2$  paint area was put into each treatment microcosm (figure 2 shows experimental phases). At that time, one microcosm, which previously had been maintained as a control, was converted to a low-residence (LoRes) organotin treatment so effects of the high-level and low-level treatments could be discerned, if necessary.

Prior to use in the treatment phases, the OMP-253 panels were leached in flowing seawater for 36 days (0.5 m<sup>2</sup> panels) and 68 days (0.1 m<sup>2</sup> panels) to reduce organotin leach rates to lower levels. While in the microcosm tanks, the panels accumulated a buff-colored film composed of bacterial slime, detritus, and diatoms. The films were not disturbed or washed from the panels except once on 25 August 1982 when all panels were gently sponged off. At that time, microcosm water samples were collected immediately before and 6 hours after sponging so organotin determinations could be made to determine whether panel leach rates were significantly influenced by sealing effects of organic layers. Surprisingly, no significant differences were found between pre- and post-cleaning leach rates.

OMP-253 panels were simply leaned against the inside tank walls where they received gentle water motion from toroidal water flow. In the HiRes-Pools, the panels were placed 0.5 m in front of the water inlet port where they received water flow equivalent to that encountered by panels in the microcosm tanks. White rubberized canvas covers were placed over the HiRes-Pools to exclude abnormal effects of ultraviolet exposure in the shallow pools and to prevent heating of the water.

Seven 14-L trays of calcareous mud containing resident infauna collected from a 10-m water depth in Kaneohe Bay, Oahu, Hawaii, were added to each microcosm tank (figure 1 shows tray dimensions and orientations). Seawater flow was then provided to the tanks for 5 months before leachate treatment to allow natural colonization and stabilization of organism populations before beginning the treatment phase of the experiment.

The second treatment phase (using 0.1 m<sup>2</sup> panel areas) lasted for 105 days during which six sets of water samples were collected for organotin analysis. At the end of the leachate treatment, OMP-253 panels were removed from the tanks, and the microcosms were allowed a recovery phase of 105 days.

Chlorophyll  $\alpha$  content of a Contr microcosm, both HiOrg microcosms, and the algal culture were monitored two to three times a week with a fluorometer to quantify levels of organic enrichment in the HiOrg treatments. Occasional samplings of water from the same water sources were made for wet analysis of chlorophyll  $\alpha$  content to provide standardization values for fluorometer readings. Dissolved organic carbon (DOC) and particulate organic carbon (POC) were measured on a few Contr and HiOrg water samples to provide an estimation of the magnitude of organic enrichment created by the algal culture additions. Additionally, enrichments of micronutrients (PO<sub>4</sub>-P, NO<sub>3</sub>-N, and NH<sub>4</sub>-N) were determined from analyses of selected water samples from Contr and HiOrg microcosms and algal cultures. Abundances and types of algal cells in cultures were generally inventoried.

## **METHODS**

### **F121/SPC-4 Comparison Experiment**

Water samples for copper analysis were collected from mid-depth over outlet ports in the microcosm tanks and were stored frozen in acid-washed 750-ml polyethylene bottles. A spectrophotometric technique (Strickland & Parsons, 1972) based on carbon tetrachloride extraction of copper complexed by sodium diethyldithiocarbamate was used for copper determinations.



Organotin water samples were also collected near tank outlet ports and were stored frozen in acid-washed 1-L polycarbonate bottles for periods not exceeding 3 days. Organotin compounds were extracted from the samples using 40 ml of chloroform or methylisobutylketone, and concentrations of tin in the extracts were determined with an atomic absorption spectrophotometer (Homer & Dooley, 1983). Some unextracted water samples were analyzed for organotin content using anodic stripping voltametry techniques (Kenis & Zirino, 1983). Because speciation of organotin compounds was not determined by either analytical technique, results are presented as organotin-Sn. Those values can be converted to approximate total organotin values by multiplying by 2.4.

### **OMP-253 Experiment**

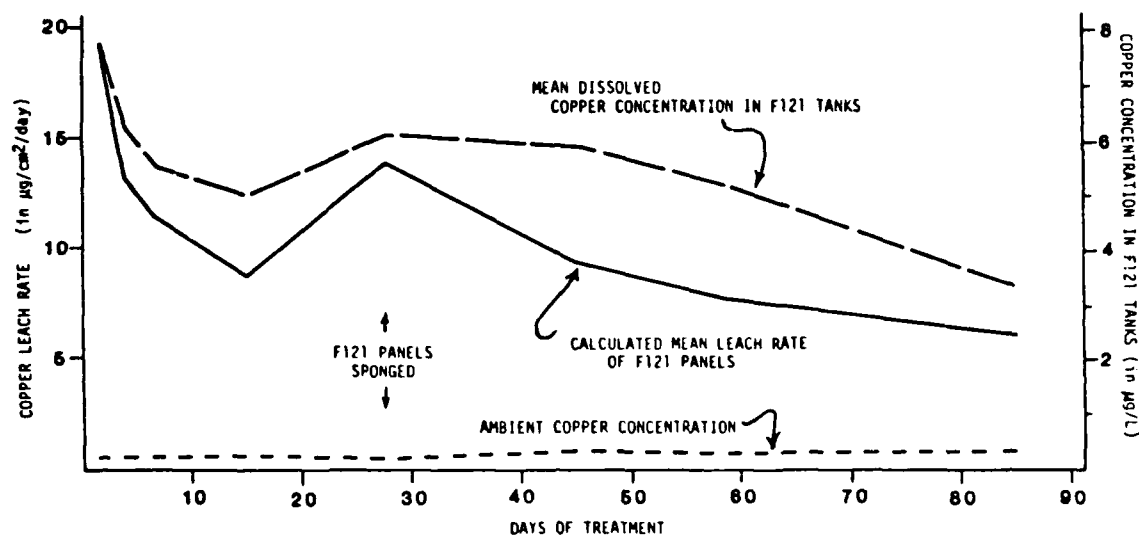
Collection and storage methods for organotin water samples were identical to those used in the F121/SPC-4 experiment. Samples were analyzed using a volatile hydride generation/atomic absorption spectrophotometry technique described in Valkirs et al. (1985). This technique determines quantities of tri-, di-, and monobutyltins, thereby providing data on the speciation status of organotins in the samples. In the follow-on presentation and discussion of OMP-253 experiment organotin data, TBT is assumed to be the organotin species that is responsible for the majority of biological toxic effects. Data are reported as the monobutyltin or dibutyltin chlorides. Tributyltin values are reported as tributyltin oxide.

Chlorophyll  $\alpha$  measurements were made with a Turner Model 111 fluorometer equipped with a flowthrough cuvette that received water from the microcosm tank inlets and outlets via an automated sampling system described in Henderson and Smith (1980). Wet analysis of chlorophyll  $\alpha$  concentrations were made with methods described in Strickland and Parsons (1972). DOC, POC, and micronutrient analyses were performed by Analytical Services, Honolulu, using techniques detailed in Strickland and Parsons (1972). Culture algal cells were identified and counted using a compound microscope and hemacytometer counting grid slide.

## **RESULTS AND DISCUSSION**

### **F121/SPC-4 Experiment**

The mean total dissolved copper concentration in the F121-treated tanks over the treatment phase was 5.6  $\mu\text{g/L}$  ( $\pm 1.43$  SD for 24 values). That value lies within the range of 2 to 14  $\mu\text{g/L}$  for copper measured in Pearl Harbor waters (R.S. Henderson, 1976 survey, unpublished data). Thus, the microcosm data indicate that F121 treatment copper levels approximated those of a harbor system they were meant to simulate. Mean copper leach rates for the F121 panels generally declined with time except for one substantial increase in leach rate following a gentle sponging of the panel surfaces (figure 4). Leach rates were calculated by multiplying copper concentrations by tank flow rates and normalizing those figures by time and area factors. The mean copper leach rate for F121 panels was 11.2  $\mu\text{g/cm}^2/\text{day}$ .



**Figure 4.** Time-series plots of mean dissolved copper concentration of tank water and mean calculated copper leach rates for antifouling panels in treated microcosms.

Two sets of dissolved copper measurements made on SPC-4 tank waters during treatments yielded a mean value of  $1.3 \mu\text{g Cu/L}$  ( $\pm 0.19$  SD for 6 values). The mean copper leach rate for those panels was calculated to be  $0.9 \mu\text{g/cm}^2/\text{day}$ .

For SPC-4 water, mean total dissolved organotin-Sn concentrations were  $0.8 \mu\text{g/L}$  ( $\pm 0.47$  SD for 7 values) by anodic stripping voltammetry analyses and  $0.7 \mu\text{g/L}$  ( $\pm 0.53$  SD for 15 values) by atomic absorption analyses. Mean tin leach rates calculated for these data sets were  $1.7$  and  $1.3 \mu\text{g/cm}^2/\text{day}$ , respectively. Eighty percent or more of the organotin measured in SPC-4 tank waters probably existed as tributyltin, as indicated by speciated samples measured in the OMP-253 experiments. Although the anion is not known, it is defined as the oxide (TBTO) because it was used as the standard.

### OMP-253 Experiment

Concentrations of TBTO from the one set of water samples taken from treated tanks during treatment phase 1 ranged from  $3.3$  to  $7.8 \mu\text{g/L}$  (table 1). Leach rate calculations for panels in the LoRes tank where leachates would be expected to be least degraded was  $16.8 \mu\text{g TBTO/cm}^2/\text{day}$ . That leach rate was about four times higher than leach rates measured for SPC-4 panels exposed to nearly identical conditions in the F121/SPC-4 experiment. Because of the high OMP-253 leach rate observed and immediate severe biological effects encountered during the  $0.5 \text{ m}^2$  panel treatments, panel areas for the second treatment phase were reduced to  $0.1 \text{ m}^2$ . Although OMP-253 paint contains only 48 percent more TBTO than SPC-4 paint, the several-fold difference in leach rates between the paints implies that the polymeric matrix of OMP-253 is dissolved or ablated about three times more rapidly than that of SPC-4. Also note that the OMP-253 was several years old at the time of use, and that matrix degradation may have resulted in unusually high release rates. More recent measurements of OMP formulations have demonstrated release rates 10 fold less.

**Table 1.** Concentrations of TBTO and dibutyltin chloride (DBTC<sub>l</sub>) for water of one tank from each 0.5 m<sup>2</sup> OMP-253 leachate treatment and one control tank.

	Contr	HiRes Tank	HiRes Pool ( $\mu\text{g/L}$ )	LoRes Tank	HiOrg Tank
TBTO	0.1	7.8	6.0	7.8	3.3
DBTC <sub>l</sub>	ND	1.0	0.8	0.8	1.0

ND — No detection

Mean TBTO concentrations for five series of water samples taken from the treated tanks during treatment phase II ranged from 0.5 to 1.8  $\mu\text{g/L}$  (table 2). The TBTO leach rate calculated for the LoRes tank 3 panel was 14.5  $\mu\text{g/cm}^2/\text{day}$ .

In the HiRes tank and pool, mean total organotin concentrations were only 29 percent and 40 percent, respectively, of the mean concentration measured in LoRes tank 3. If it is assumed the OMP-253 panels in the HiRes-Pool were leaching organotin at the same rate as panels in the LoRes tanks, then a substantial amount of dissolved organotin was being removed from waters in the HiRes containers. Adsorption onto container walls and sediments and adsorption/uptake by plankton are suggested organotin loss mechanisms in the HiRes containers.

Unexpectedly, mean organotin concentrations in the HiOrg tank were not significantly lower than those of the LoRes (no organic enrichment) tanks. As water samples were not filtered before analysis, much of the organotin in HiOrg samples was thought to have been derived from organotin loosely adsorbed onto particulate organics (largely the added phytoplankton). However, results of a single set of analyses on unfiltered and GF/C-filtered water samples from the OMP-253 treatments provided no evidence of binding of organotins to particulates retained by filters. Particles smaller than semicolloidal size, however, are not retained by GF/C filters and, therefore, are possible binding agents for organotins. Future work should examine organotin content of samples with and without subcolloidal fractions removed.

Degradation of TBTO was apparently highest in the HiOrg tank where the mean amount of degradation products (DBTC<sub>l</sub> and MBTC<sub>l</sub>) was 37 percent of total organotin. In HiRes tank 1, degradation products were 25 percent of total organotin, and for the HiRes pool and LoRes tank 6 degradation products were 18 percent and 17 percent, respectively. Although these values resulted from only six analyses per tank, the general trend of low-to-high degradation noted was low residence time: high residence time: high organic.

**Table 2.** Organotin concentrations in 0.1 m<sup>2</sup> OMP-253 treatment tanks and a control tank.

Water Source	Date	MBTC $\mu$ ( $\mu$ g/L)	DBTC $\mu$	TBTO	Mean TBTO ( $\pm$ SD)
HiRes	7/19	0.1	0.2	0.2	
Tank 1	8/9	ND	ND	0.2	0.5
	8/25 (a)	0.2	0.1	0.9	
	8/25 (b)	0.2	ND	0.9	( $\pm$ 0.38)
	8/30	ND	ND	0.2	
HiRes	7/19	0.2	0.3	0.8	
Upstream	8/9	ND	0.1	0.3	0.7
Pool	8/25 (a)	ND	ND	0.9	
	8/25 (b)	0.1	ND	1.2	( $\pm$ 0.37)
	8/30	0.1	ND	0.4	
LoRes	7/19	0.1	0.4	2.3	
Tank 6	8/9	ND	0.7	1.7	1.2
	8/25 (a)	ND	ND	1.0	
	8/25 (b)	0.1	ND	0.9	( $\pm$ 0.80)
	8/30	ND	ND	0.2	
LoRes	7/19	0.1	0.7	3.8	
Tank 3	8/9	0.1	0.2	1.9	1.8
	8/25 (a)	0.2	0.1	1.3	
	8/25 (b)	0.2	0.3	1.0	( $\pm$ 1.18)
	8/30	0.1	ND	1.0	
HiOrg	7/19	0.1	1.2	1.5	
Tank 8	8/9	0.1	0.2	2.2	1.7
	8/25 (a)	0.3	1.2	1.9	
	8/25 (b)	0.2	0.6	1.5	( $\pm$ 0.36)
	8/30	0.3	0.3	1.3	
Contr	7/19	ND	ND	ND	0.07
Tank 5	8/25	ND	ND	0.1	
	8/30	ND	ND	0.1	( $\pm$ 0.058)
ND = No detection					
MBTC $\mu$ = Monobutyltin chloride					
(a) = Sample taken before panels were gently sponged off					
(b) = Sample taken 6 hours after panels were sponged off					

Measurements made from 23 April to 21 September 1982 indicated that mean chlorophyll  $\alpha$  level in HiOrg microcosms was about 10 times higher than ambient (Contr) concentrations (table 3). The mean chlorophyll  $\alpha$  concentration for HiOrg microcosms was 1.16 mg/m<sup>3</sup>, which is very close to the mean chlorophyll  $\alpha$  concentration of 1.23 mg/m<sup>3</sup> reported by Smith et al. (1981) for sewage-affected areas of Kaneohe Bay. Similar concentrations would be expected in tropical and subtropical nutrient/sewage-enriched Navy harbors, such as Pearl Harbor and Subic Bay.

Results of POC and DOC analyses (table 4) showed that POC concentrations correlated well with chlorophyll  $\alpha$  contents of the same samples. On the other hand, DOC concentrations were not measurably affected by plankton abundance, and Contr and HiOrg tanks were not significantly different in DOC content.

Dissolved phosphate and nitrate concentrations in HiOrg tanks were increased several times above ambient levels by addition of algal culture water (table 4). Ammonium and silicon levels in HiOrg tanks were not significantly different from Contr levels. Based on computations of amounts of dissolved nutrients that should have been present in the HiOrg microcosms according to flow rates and addition rates of culture, microcosm communities consumed about 38 percent of the added P<sub>04</sub>-P and about 31 percent of the added N<sub>03</sub>-N. The mean N/P ratio of consumed P<sub>04</sub>-P and N<sub>03</sub>-N was 9.5. These uptake rates and ratios are very similar to those observed in a previous microcosm experiment using similar benthic communities and dissolved nutrient additions (Henderson & Smith, 1980). The major nutrient consumers in HiOrg microcosms were probably the thick carpets of green algae (*Cladophora socialis*) that developed in those tanks during leachate and phytoplankton addition treatments (section 2).

**Table 3.** Chlorophyll  $\alpha$  concentrations for Contr and HiOrg microcosms and added culture. Also given are the culture cell counts, approximate culture species composition and mean chlorophyll  $\alpha$  enrichment factors for HiOrg treatments.

Sample	mg chlorophyll $\alpha$ /m <sup>3</sup>				Culture count Cells/ml X10 <sup>6</sup>	% Comp. Diatom/ Blue-green	Enrichment Factor*
	Contr Tank 5	HiOrg Tank 8	HiOrg Tank 9	Culture			
4/23	0.08	1.15	1.46	122.5	-	-	16.3
4/23	0.13	0.97	0.72	114.1	1.46	33/63	6.5
8/25	0.11	1.83	2.21	188.5	3.55	20/?	18.4
8/31	0.16	2.03	3.02	185.1	2.60	39/?	15.8
9/3	0.15	0.70	2.82	18.0	1.70	35/54	11.7
9/8	0.10	0.80	1.37	176.7	1.96	35/46	10.9
9/13	0.16	0.99	0.73	123.4	0.74	30/43	5.4
9/15	0.05	0.11	0.12	10.3	0.61	56/39	2.3
9/20	0.06	0.44	0.50	15.6	1.02	1/83	7.8
9/21	0.11	0.85	0.43	85.1	1.46	29/67	5.8

Mean enrichment factor = 10.1

\* = Computed as mean chlorophyll  $\alpha$  for tanks 8 and 9 divided by chlorophyll  $\alpha$  for tank 5.

**Table 4.** Particulate and dissolved organic carbon and chlorophyll  $\alpha$  content of Contr and HiOrg microcosm water.

Water Source	Chlorophyll $\alpha$ (mg/m <sup>3</sup> )	POC (mg/L)	DOC (mg/L)
Contr-Tank 5	0.05	0.094	0.87
HiOrg-Tank 8	0.11	0.149	0.83
HiOrg-Tank 9	0.12	0.177	0.78

In comparing biological data (section 2) and chemical measurements from OMP-253-treated tanks and pools, organotin concentrations were found to be highest in microcosms containing the highest benthic algae coverage and were lowest in containers with the lowest algal coverage. This relationship strongly indicates that organotins are not significantly adsorbed or assimilated by live plant material, but instead are removed from the water column by contact with inorganic substrates such as sediments. Such a model is consistent with laboratory observations showing that organotins have a very strong affinity for sediments (e.g., Dundee et al., 1980; Dooley & Homer, 1983). If the affinity of live flora for organotins is indeed substantially lower than sediment affinity for organotins, the presence of extensive algal coatings over inorganic substrates would be expected to inhibit sediment uptake from the water column.

Measurements of quantities of suspended particulate inorganic material (PIM) were not made on microcosm waters. The potential role of PIM in the adsorption of organotin compounds should be examined in future studies.

## SECTION 2.

### POPULATION STRUCTURES OF BENTHIC MICROCOSMS EXPOSED TO ANTIFOULING LEACHATES

#### INTRODUCTION

Changes in the population structure of marine communities are an important measure of environmental impact of pollutants on an ecosystem. Marine flowthrough microcosms of intermediate size are particularly well suited for population response studies because complex communities of plants and animals can be sustained indefinitely with normal foods and a source of natural recruitment provided by supply seawater. Continuous addition of pollutant to established microcosms allows gradual and subtle changes in communities to be documented as various organisms increase or decrease in abundance.

The primary purpose of the efforts described in this section was to examine the response of soft-bottom infauna and hard-bottom epibiota to long-term exposure to antifouling leachates. Results are presented for two long-term experiments. Experimental design and water chemistry of the experiments are described in section 1.

#### METHODS

##### F121/SPC-4 Experiment

To supplement naturally-recruited infauna in the microcosm sediments, specific organisms were collected from the field and introduced to the microcosms. The organisms added per tank were 12 *Bathygobius fuscus* (gobiid fish), 6 *Alpheus heeia* (snapping shrimp), and 2 *Holothuria edulis* (sea cucumber). Resultant communities in the microcosms are typical of those found in inshore Hawaiian environs (figure 5).

Two boxes of mud and two boxes of rubble/mud from each microcosm were sieved through 0.5-mm mesh to sample infauna at the end of the treatment phase. Sieved sediments were returned to the boxes and microcosms from which they had been removed. Sediment boxes not sampled after the treatment phase were sieved at the end of the recovery phase. Biota retained by sieving were preserved in 15-percent Formalin and were later counted and identified to at least phylum level.

Observations on the abundance and distribution of macrobiota, animal burrows, and algae in the microcosms were made periodically during treatment and recovery phases. It was also noted whether organisms were conspicuously lacking or missing in microcosms.

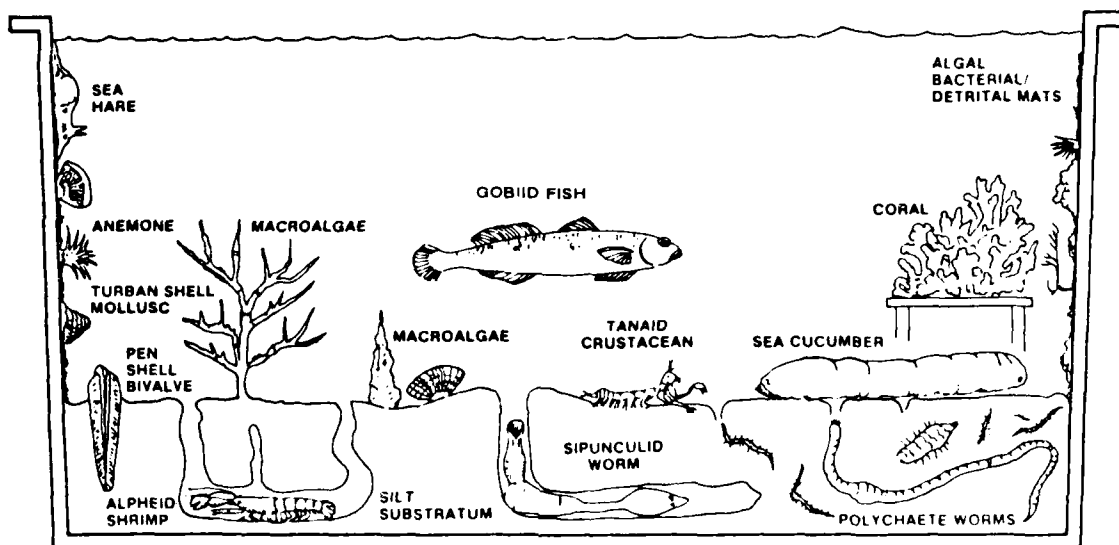


Figure 5. Organisms typical of soft-bottom microcosm communities.

### OMP-253 Experiment

Microcosm sediment boxes (as described for the F121/SPC-4 experiment) were filled with calcareous mud including resident infauna collected from a 10-m depth in Kaneohe Bay. Seawater flow was provided to the tanks for 148 days prior to treatment to allow colonization and stabilization of infauna. Seven gobiid fish (*Bathygobius fuscus*) and two sea cucumbers (*Holothuria edulis*) were added to each microcosm from field-collected stock during the pre-treatment phase.

A single tray of mud from each microcosm was sieved for infaunal inventory near the end of recovery phase I, and three trays of mud from each tank were sieved at the termination of treatment phase II and recovery phase II. Collection and analysis methods for infaunal samples were the same as those used for the F121/SPC-4 experiment.

## RESULTS AND DISCUSSION

### F121/SPC-4 Experiment

Benthic algae were the first microcosm organisms to exhibit obvious shifts in abundance in an apparent response to antifouling leachate treatments. By the 24th day of the treatment phase, algal mats on the walls and bottom substrate in the SPC-4 tanks were dominated by a low-diversity community composed primarily of *Cladophora socialis*, a filamentous green alga covered with a coating of diatoms. This tufted algal mat was generally 0.7 to 1.5 cm thick and had a tan overall appearance due to diatom coatings. In comparison, algal mats in the control and F121-treated microcosms were thin and patchy and consisted of diverse mixtures of blue-green, green, brown, and red algae. Benthic diatoms were common in control tanks, but were much more abundant in F121 tanks where they occurred as a thin, snow-like coating over attached algae.



Similar increases in diatom abundance associated with heavy metal stress have been observed in a previous experiment (Evans, 1977). Copper concentrations of 10 and 100  $\mu\text{g/L}$  maintained for several weeks produced moderate and dense (respectively) growth of diatom-dominated mats. Average thickness of mats formed in the 100- $\mu\text{g/L}$  Cu treatment was equivalent to that encountered in the 0.8- $\mu\text{g/L}$  organotin-Sn levels in SPC-4 treatment.

The increased aggregation of diatoms was attributed to potential binding effects of a higher than normal secretion of mucilage by the diatoms in response to copper poisoning. Mucilage production may provide an absorptive sheath of organic material that could selectively favor survival of diatoms by protecting them from excessive copper or organotin exposure. Flocculent material, or "marine snow," often reported in harbors such as Pearl Harbor (Evans & Morris, 1974), may be composed at least partially of aggregations of diatoms and detritus caused by enhanced mucilage production generated by heavy metal exposure.

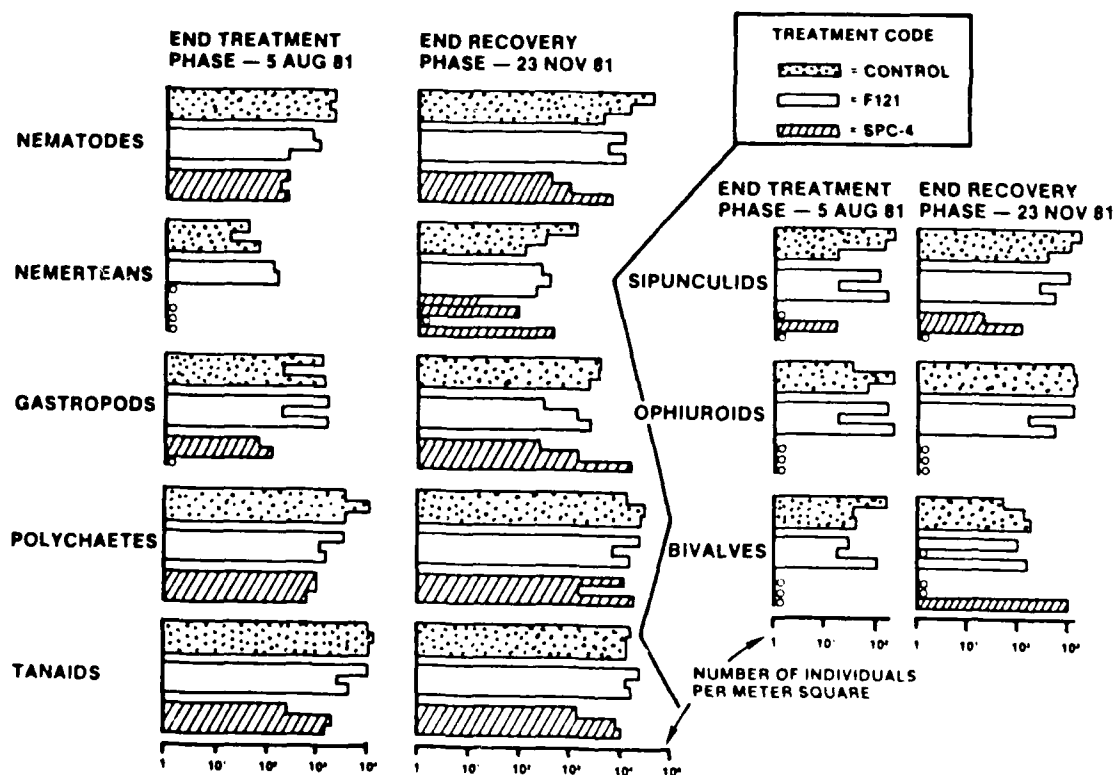
Algal mats, as described in the microcosms on treatment day 27, remained essentially unchanged through the remainder of the treatment period. During the same interval, macroalgae were found to be common in the control and F121 tanks, but were rare in the SPC-4 treatments. A few thalli of *Padina japonica* (fleshy brown alga) and *Acanthophora spicifera* (a branching brown alga) were found in the SPC-4 tanks. Both are species found to be particularly tolerant of copper treatment in previous experiments (Evans, 1977). Other macroalgae that appeared normal but in sparse amounts were an encrusting calcareous (red) alga and the green algae *Dictyosphaeria versluyii* and *Valonia ventricosa*.

Visual inventories at the termination of panel treatments revealed no major differences in animal populations between control and F121 microcosms. Several animal species, however, were severely reduced in abundance in SPC-4 tanks. Between days 23 and 42 of the treatment phase, all *Aiptasia pulchella* (common anemones) died in the SPC-4 tanks. This organism was very abundant in control and F121 tanks, and its susceptibility to SPC-4 toxins was unexpected because it is present even in relatively highly polluted areas of Pearl Harbor.

A few sea hares (*Stylocheilus longicauda*) were occasionally seen in SPC-4 tanks during treatment phase, but never occurred in large numbers in those microcosms as was the case in control and F121 tanks. High sensitivity of sea hare larvae to SPC-4 leachates may have caused the low abundance of this organism in SPC-4 tanks as indicated by the fact that adults appeared healthy and normal.

One of six *Holothuria edulis* (sea cucumber) died in the SPC-4 tanks, two developed necrotic lesions, and all lost weight during the treatment phase. Sea cucumbers in control and copper tanks all survived and steadily increased in size. Readily visible animals that appeared normal in SPC-4 microcosms included *Bathygobius fuscus* (gobiid fish), palaemonid shrimp, *Thalamita* spp. (swimming crabs), zoanthids (soft corals), and several species of encrusting sponges.

Species abundances in all eight phyla of cryptic infauna sieved from the microcosm sediments at the end of the treatment phase were consistently lower in SPC-4 treatments compared to F121 and control tanks (figure 6). Five of the eight phyla inventoried in SPC-4 tanks showed near complete recovery with abundances similar to controls; whereas nemerteans, sipunculans, and bivalves had recolonized only one or two tanks and ophiuroids reappeared in none of the SPC-4-treated environs.



**Figure 6.** Infauna abundance in various treatment tanks of the F121/SPC 4 leachate experiment at ends of treatment and recovery phases.

Based on the abundance of infaunal groups in the microcosms at the termination of the treatment and recovery phases, a hierarchy of increasing sensitivity of SPC-4 leachates would establish tanaids and polychaetes as the most resistant organisms, with gastropods, nemerteans, and sipunculans showing intermediate sensitivity, and bivalves and echinoderms (ophiuroids and holothurians) being the most intolerant. On the other hand, infauna exposed to F121 (copper) leachates showed no substantial differences in abundances compared to controls at the end of treatment or recovery phases.

Approximately one-third of the benthic organisms inventoried in control microcosms are also found in inshore-polluted environs typical of Pearl Harbor (Grovhoug & Guinther, 1974; Grovhoug, 1979; Henderson & Smith, 1980). About one-third of the "polluted environs" organisms sustained population reductions under organotin treatment, indicating they were generally not more tolerant of organotins than were "clean water" organisms.

### OMP-253 Experiment

After 50 hours of exposure to leachates from a 0.5 m<sup>2</sup> OMP-253 panel, nearly all visible organisms in treatment tanks were negatively affected. The normally abundant *Aiptasia pulchella* (common anemone) had disappeared completely in all treatment tanks, and all *Holothuria edulis* (sea cucumber) had stopped feeding. All visible *Stylocheilus longicauda* (common sea hare) were dead in the LoRes and HiOrg tanks, and only one sea hare was living in each of the HiRes tanks. Individuals of another species of sea hare (*Dolabrifera dolabrifera*) that occurred only in the LoRes tanks were alive, but stressed as manifested by pigmentation changes and cessation of feeding. Pen shell bivalves (*Pinna muricata*) were killed in all leachate treatments.

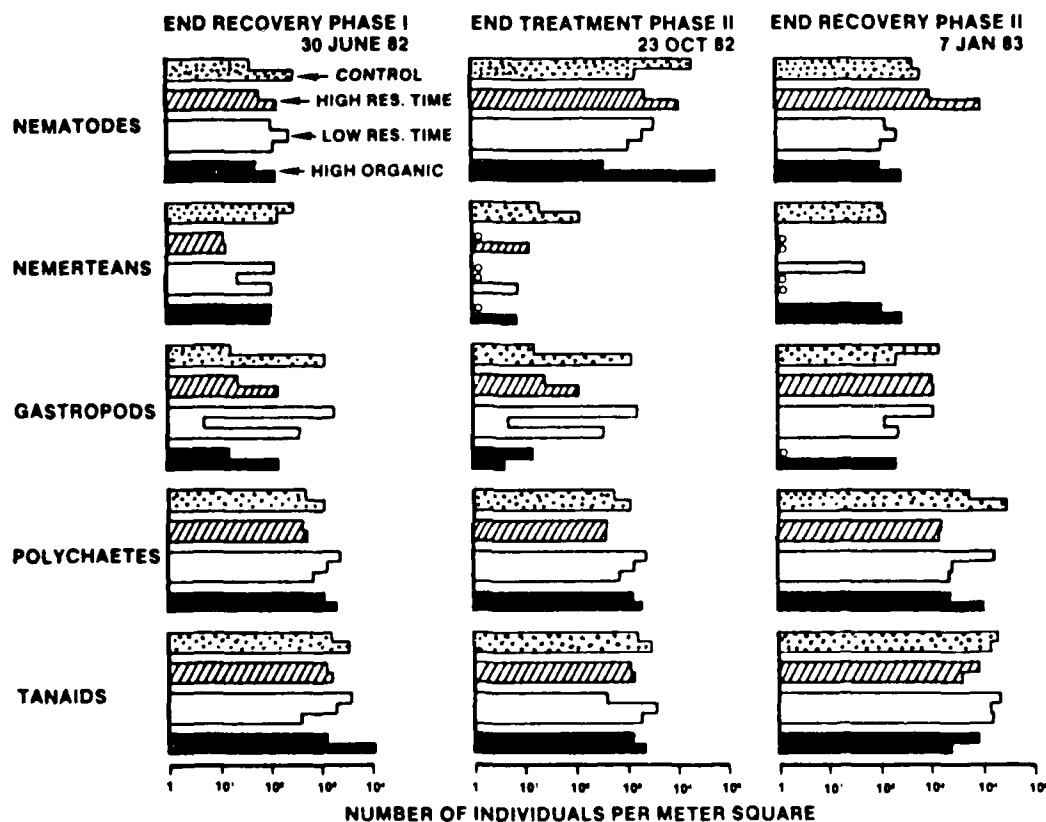
Gobiid fish (*Bathygobius fuscus*) appeared normal and active in all treatments. Sediments were pumped from burrows at normal rates by alpheid snapping shrimp. One cirratulid polychaete worm (species unidentified) was seen with feeding arms distended normally in a HiOrg tank.

After 2 to 4 days of exposure to 0.5 m<sup>2</sup> panel leachates, most thalli of *Acanthophora spicifera* (red alga), *Caulerpa racemosa*, and *Caulerpa taxifolia* (green algae) were dead. All other macroalgae appeared normal. Mats of microalgae in leachate tanks had decreased coverages of dark algal films (blue-green algae) and higher than normal quantities of benthic diatoms for about 2 weeks after the first phase of exposure.

Most flora and fauna in leachate-treated microcosms had returned to normal abundance and appearance within 3-1/2 weeks after exposure was terminated. Individuals of *Holothuria edulis* (sea cucumber), however, steadily lost weight and did not resume feeding. Those individuals were removed from the microcosms and were replaced with newly collected sea cucumbers. Infaunal inventories performed 2 months after the 0.5 m<sup>2</sup> panel treatments (figure 7) showed population abundances of previously-treated microcosms to be essentially the same as controls.

One week after the beginning of treatment phase II (0.1 m<sup>2</sup> panel areas), biota observations were normal in HiRes tanks, whereas other leachate treatments exhibited some negative effects. In LoRes tanks, only a few bleached (stressed) anemones remained, and all visible sea hares were immobile or dead. Sea cucumbers in those treatments had stopped feeding, and 70 percent of *Caulerpa* spp. (green algae) tissue was yellowed and dead. The condition of organisms in the HiOrg treatments was generally the same. However, 12 sea hares in one tank were mobile and normal.

Three days later, leachate effects became evident in HiRes tanks as sea cucumbers stopped feeding and anemones were found bleached with withdrawn feeding tentacles. Additionally, small gastropod mollusks normally common in the microcosms were only present in control tanks and one of the HiRes tanks.



SMALL CIRCLES DENOTE NO INDIVIDUALS FOUND

**Figure 7.** Infauna abundance in various treatment tanks of OMP-253 leachate experiment at ends of recovery phase I, treatment phase II, and recovery phase III.

After 4 weeks of leachate exposure, HiRes tanks displayed the lowest overall levels of toxic effects. Two to three anemone and sea hare specimens were present in the HiRes treatment tanks. These same species had been eliminated in all other leachate treatments. Alpheid shrimp burrow patterns remained normal in all tanks, indicating unchanged abundances. Gobiid fish in all leachate microcosms looked emaciated in comparison to control gobies, suggesting that less of their preferred food (microcrustaceans) was available or they had simply decreased their feeding activity. Sea cucumbers continued not to feed in leachate tanks, and at least one sea cucumber had died in each treatment group.

When compared to controls, algal films and mats in HiRes and LoRes tanks were considerably reduced in coverage and diversity of species after 4 weeks of exposure. Moderate to thick growths of filamentous green algae (*Cladophora socialis*) covered with diatoms appeared in all leachate tanks, and were particularly abundant in HiOrg microcosms where they formed long stringy masses and covered nearly all available substrate. Although abundances and diversity of macroalgae were reduced in some leachate tanks, it was not clear if the reductions were caused by direct toxic effects or competition with *C. socialis*. At least 12 species of common macroalgae were observed in various leachate microcosms in the course of treatments (table 5). Only four species of fleshy macroalgae were found in SPC-4 leachate treatments during the F121/SPC-4 experiment. Strong algicidal effects of the SPC-4 leachate were probably caused by the cuprous thiocyanate component of that paint.

**Table 5.** Macroalgae observed in OMP-253 leachate microcosms during treatment phase II.

Green Algae	Brown Algae	Blue-Green Algae
<i>Bornatella sphaerica</i>	<i>Dictyota acutiloba</i>	<i>Schizothrix</i> sp.
<i>Caulerpa racemosa</i>	<i>Padina japonica</i>	
<i>Chaetomorpha antennina</i>	<i>Sargassum</i> sp.	
<i>Cladophora socialis</i>		
<i>Enteromorpha</i> sp.	Red Algae	
<i>Neomeris annulata</i>	<i>Acanthophora aspicifera</i>	
<i>Ulva</i> sp.		

The composition and abundance of biota in the microcosms remained essentially unchanged during the 4 to 15-week treatment phase interval. During this period, amounts of *C. socialis* algae and diatom coverage increased in most leachate tanks and spirorbid polychaete tube worms settled in abundance on open, shaded substrates in HiOrg tanks. Additionally, a few individuals and egg masses of *Dolabrifera dolabrifera* (sea hare) were seen in a HiRes and a LoRes tank. Organisms characteristic of polluted or unpolluted waters were not differentially affected by organotin leachates, as was observed in the F121/SPC-4 experiment.

Nemertean worms were the only infaunal group inventoried that showed obvious reductions in treated populations compared to controls (figure 7). Because mixed rubble and mud sediments were not used in the OMP-253 experiment, sipunculans, ophiuroids, and bivalves were not as common in microcosms of that experiment as they were in the F121/SPC-4 experiment. However, at the beginning of the OMP-253 treatment phase, several large dead ophiuroids and bivalves were observed in treatment tanks and no live organisms of those phyla were seen in any leachate tanks.

Recovery of microcosms occurred quickly after termination of the OMP-253 treatment, as was the case in posttreatment SPC-4 leachate tanks. After only 2 weeks of recovery, *Aiptasia pulchella* (common anemones) had recolonized all HiRes and LoRes tanks, and *Cladophora socialis*/diatom algal crops in all leachate-treated tanks had declined in abundance and showed signs of senescence. Anemones were reestablished in HiOrg tanks after 6 weeks of recovery, and *Stylocheilus longicauda* (sea hares) were common in all microcosms. *C. socialis* and other green algae that

were abundant under leachate treatments were gradually displaced by other algal types, especially blue-green algae.

After 104 days of recovery, infaunal group abundances were not significantly different from their status at the end of treatment phase II (figure 7). Nemertean abundances had returned to control levels in HiOrg tanks, but no individuals were present in HiRes tanks and in two out of three LoRes tanks. One HiOrg tank contained no gastropods.

The toxicity of residual leachates may have exerted some direct effects of the posttreatment survivability of sensitive fauna, especially for larval stages. However, fluctuations in amounts of specific predators and foods during recovery/recolonization periods would very likely also play an active role in governing abundances of certain organisms. Depressed abundances of some phyla seen in recovery phases may, therefore, be only artifacts of temporary factors involving biotic changes during seral succession.

## General Discussion

Effects on fauna of leachates derived from equivalent areal paint surfaces were substantially greater for OMP-253 compared to SPC-4 paints. Effects reflected the leachate concentrations measured in the microcosms. Many species of plants and animals characteristic of both inshore-polluted and offshore-clean environments were excluded from the microcosms by chronic exposure to TBTO concentrations in the 0.5- to 2.0- $\mu\text{g/L}$  range. SPC-4 paint leachates had the highest relative effects on algae, probably because of the cuprous thiocyanate toxin released by that paint.

Animals exposed on open surfaces (epifauna) were more strongly affected by leachates than animals living in the sediment (infauna). This difference suggests that infauna may somehow be better protected from leachates (possibly by absorption of toxins onto sediments) or may be tolerant of dissolved toxins. For most harbor situations, where soft sediments usually comprise about 80 to 90 percent of the bottom, this finding implies that the overall biological impact would be considerably less than in a typical microcosm where less than 40 percent of bottom substrate consists of soft bottom material.

Furthermore, shallow water epifauna or fouling communities in low-circulation harbors tend to be dominated by animals that are encased in hard shells or leathery tunics and are capable of closure of water circulation openings for periods up to several hours. Such a mechanism enables those organisms to isolate themselves from the surrounding water column when certain pollutants, such as oil, freshwater, and copper, are present (e.g., Cheshier, 1975). Common groups of fouling organisms that fall into this category include oysters, tunicates, tubeworms, vermetid worms, and barnacles. If these organisms are also capable of sensing and isolating themselves from pulses of organotin-rich water, harbor-type fouling communities would likely be considerably more resistant to antifouling leachate toxins than "open-coast" epifauna. Site specific flowthrough bioassay tests at Pearl Harbor are planned to examine the responses of harbor-fouling communities to organotin leachates.

A major factor limiting assessment of the realistic potential impact of organotins in natural environs is the sparsity of meaningful data relating to the persistence and breakdown of organotins in seawater. Organotin concentrations used for chronic microcosm and laboratory experiments were selected to reflect field concentrations based on harbor water volumes and flushing characteristics and projected paint application on ship hulls. Unfortunately, such models must assume a conservative behavior (no breakdown or removal from water column) of organotins in natural waters. The fact that organotins do *not* behave conservatively (i.e., they *are* degraded or removed from the water column at significant rates) has been indicated, not only by lower concentrations of leachate seen under the "aging" conditions of the OMP-253 experiment, but also by data from a variety of studies that have recorded high adsorption rates of organotins in association with organic and inorganic substrate (e.g., Dooley & Homer, 1983).

Mean concentrations of tributyltin measured in two San Diego Harbor marinas, using the same analytical techniques as those used in the OMP-253 experiment, were only 5 to 20 percent of those measured in the second treatment phase of the OMP-253 microcosm experiment (Valkirs et al., 1985). Although only 15 to 20 percent of the craft in those marinas were estimated to be using organotin antifouling paint, the ratio of total organotin-painted hull area present to the volume of each marina is similar to the ratio that would be encountered in a larger body of water in a harbor containing commercial and Navy ships. If this is the case, then the substantial negative biological effects seen in the microcosm experiments should be used only as "worst case" indicators of potential effects in field situations. These worst case conditions would likely only prevail near point sources of organotin output, such as ship aggregations and outfalls of piping systems painted with organotin compounds, or in poor circulation water bodies containing large numbers of vessels using high-release rate organotin antifouling paints.

## **SECTION 3.**

# **METABOLIC RESPONSES OF BENTHIC MICROCOSMS EXPOSED TO ANTIFOULING LEACHATES**

## **INTRODUCTION**

Stress-induced shifts in the overall health and structure of marine bottom communities, especially in microbial and cryptic components, can be difficult and tedious to assess using conventional techniques. In recent years, measurements of community-level metabolism have provided useful indications of functional responses to effects of pollutants. For example, the amounts of oxygen produced and consumed by a community over daily cycles can be used to estimate the degree of dominance and performance by producers (plants) or consumers (animals) and to determine total amounts of organic material produced or consumed in the system. Stressed communities will normally exhibit marked changes in patterns of oxygen flux as compared to unstressed (control) communities, and metabolic responses will commonly be evident before other structural, behavioral, or health symptoms.

Microcosms consisting of replicate marine bottom communities housed in flowthrough outdoor aquaria have been used successfully for chronic effect evaluations of several pollutants including heavy metals, depressed salinity, and elevated nutrients (e.g., Evans, 1977; Smith et al., 1979; Henderson & Smith, 1980). Measurement of community metabolism in the course of such experiments is a relatively simple and precise process because of the contained and controlled conditions surrounding the communities. Dissolved oxygen and nutrient concentrations are simply determined at periodic intervals for water as it enters and leaves the microcosms, and those concentrations are multiplied by flow factors to provide actual fluxes of metabolic products. Comparisons of metabolic and biologic data from treated microcosms with that of untreated microcosms are used to interpret the differences.

This section describes the metabolic aspects of two microcosm experiments designed to examine the fate and biological effects of dissolved leachates from three types of antifouling paints. Other experimental design, water chemistry, and biotic aspects of these experiments are described in other sections of this report.

## **METHODS**

### **F121/SPC-4 Experiment**

The biological composition of microcosms was monitored periodically through observations and sampling (section 2). Calcareous bottom sediment in each of the microcosms was contained in 10-cm-deep trays and consisted of four trays of mud and three trays of mud and rubble mix.



To monitor oxygen production or consumption by the microcosm communities, the dissolved oxygen concentration of water from tank inlets and outlets was measured at 10- to 14-day intervals during the first half of the treatment phase and once near the end of the treatment and recovery phases. An automatic water shunting and measurement/data logging system described in Henderson and Smith (1980) was used in conjunction with a polarographic Yellow Springs Instruments Model 57 oxygen meter for oxygen monitoring. Instantaneous rates of oxygen flux were computed by subtracting inlet oxygen values from outlet oxygen values and multiplying the resultant values by tank flow rates, which were generally measured daily.

### **OMP-253 Experiment**

Observations of epibiota through the experiment showed that magnitudes of toxic effects in the various treatments were correlated with mean organotin concentrations seen in the second treatment phase (section 2). Calcareous mud substrate was contained in 10-cm-deep trays and covered most of the bottom areas of the microcosms.

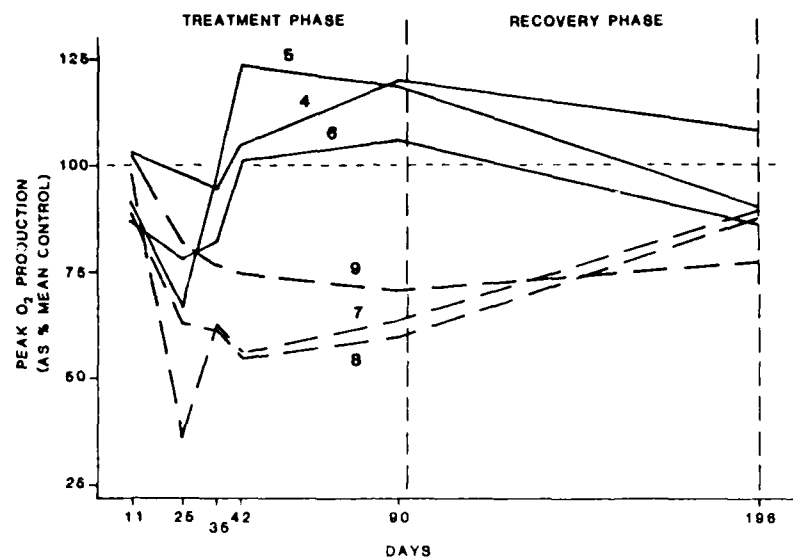
At intervals ranging from 4 to 90 days, oxygen measurements were made twice hourly on the tank inlet and outlet sources over 1- to 4-day periods. Oxygen monitoring was accomplished using the same automated system described for the F121/SPC-4 experiment. Oxygen data were stored automatically on magnetic tape in the course of monitoring runs and were later subjected to a computer program that plotted oxygen flux versus time curves for each tank. Areas above and below zero flux levels were integrated by computer to reveal total diurnal production or consumption of oxygen by the microcosms.

## **RESULTS AND DISCUSSION**

### **F121/SPC-4 Experiment**

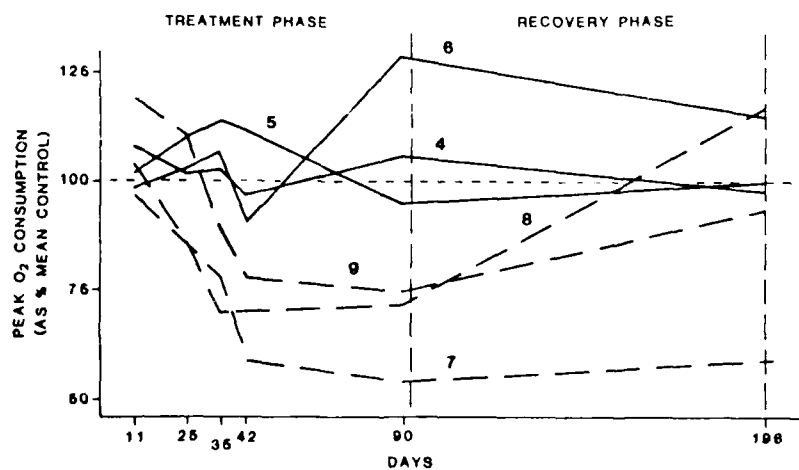
Peak oxygen production ( $P_p$ ) in F121 tanks revealed moderate declines in the first 3 weeks of leachate treatment and then increased abruptly at the middle of the treatment phase to levels of 101 to 124 percent of control values. After about 15 weeks of recovery,  $P_p$  in the F121 tanks had returned to near-control levels (figure 8). Those trends generally followed the abundance of benthic diatoms, the presence of which apparently was favored by elevated copper and organotin concentrations (section 2).

Peak oxygen consumption or respiration ( $R_p$ ) in F121 treatments remained near control levels for the duration of the experiment in two tanks, but was high (128 percent of control) at the end of the treatment and recovery phase in the third tank (figure 9). Dead organic material derived from senescent algae (largely benthic diatoms that had increased in abundance through the early treatment phase) may have caused this elevated  $R_p$  in the third tank by providing an abundance of food for microbial blooms.



4,5 and 6 = F121 leachate treatments; 7, 8 and 9 = SPC-4 leachate treatments.

**Figure 8.** Peak O<sub>2</sub> production for F121 and SPC-4 leachate treatment microcosms.



4,5 and 6 = F121 leachate treatments; 7, 8 and 9 = SPC-4 leachate treatments

**Figure 9.** Peak O<sub>2</sub> consumption for F121 and SPC-4 leachate treatment microcosms.

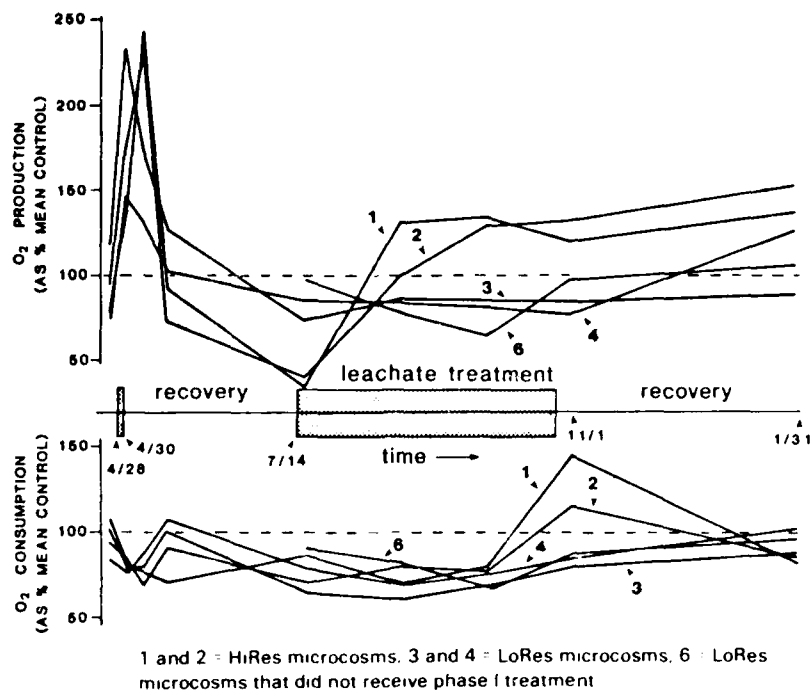
R<sub>p</sub> declined steadily in SPC-4-treated tanks through the first half of the treatment phase and was 54 to 75 percent of the mean control level at the end of the treatment phase (figure 9). The low R<sub>p</sub> in the treatments was likely a result of toxic effects of the combined SPC-4 leachates on the abundance and metabolism of both autotrophs (plants) and heterotrophs (animals). Deleterious effects on those components were clearly evident in observations of biota and sediment throughout the experiment (sections 2, 4, and 5).

Peak respiration had returned to levels at or above the control level in two SPC-4 tanks at the end of the recovery phase (figure 9). R<sub>P</sub> remained low in the third tank after recovery, probably due to delayed recolonization by heterotrophic microbes as indicated by the fact that the P<sub>p</sub> (and therefore autotrophic standing crop) had recovered to a near-control level after recovery (figure 8). A lack of sediment turnover by infauna in that tank may have caused anoxic conditions in the sediments to persist, thereby discouraging growth of normal, aerobic microbial populations.

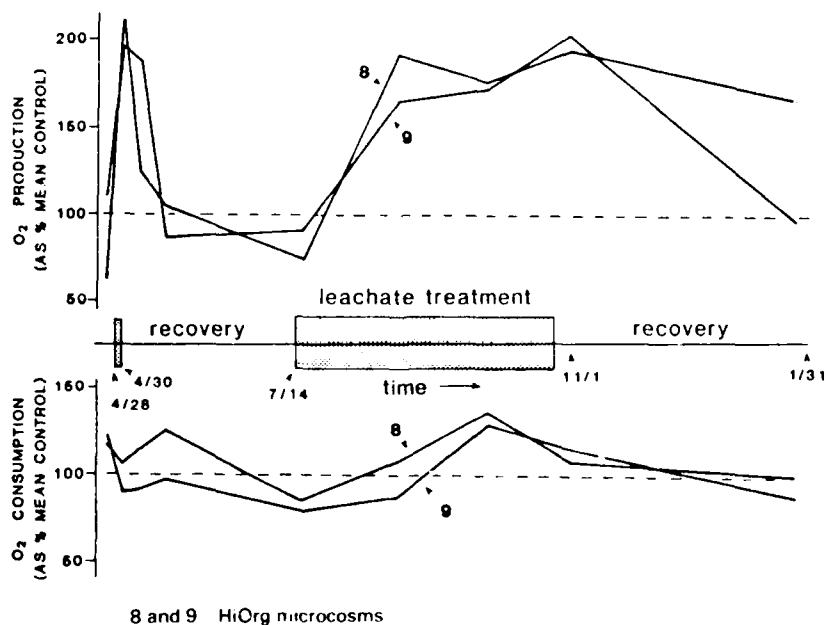
### OMP-253 Experiment

All microcosms exposed to leachates in the high-level treatment phase experienced high magnitude spikes of diurnal oxygen production (P<sub>d</sub>) that peaked 2 to 4 days after termination of treatment and then fell back to levels at or below the control (figures 10 and 11). The spikes were in part artifacts of a simultaneous decline in respiration in those same treatments (figures 10 and 11), which would cause net daytime oxygen production to increase. However, only relatively small portions of the P<sub>d</sub> spikes can be attributed to decreased respiration rates. The actual increases in oxygen production seen imply that a dramatic short-term physiological/metabolic effect occurred in algae dosed with TBTO leachate concentrations of 3.3 to 7.8 µg/L. Because of the short duration of the first treatment phase, it was not possible to determine whether elevated oxygen production could be sustained for long periods of time under those concentrations of organotin or whether the plant communities would eventually succumb to toxic effects.

At the end of recovery phase I, the P<sub>d</sub> in each treatment tank was lower than the mean control level. After 75 days of treatment phase II, however, P<sub>d</sub> in both HiRes tanks that received "aged" leachate had steadily increased to about 130 percent of the control level; whereas, P<sub>d</sub> in the three "fresh" leachate tanks remained at essentially unchanged levels only slightly to moderately lower than the control level. Throughout recovery phase II, the P<sub>d</sub> of aged leachate treatments increased slightly and the P<sub>d</sub> of one of the fresh leachate tanks increased; whereas P<sub>d</sub> in the other remained the same. These P<sub>d</sub> patterns through treatment and recovery phases showed less suppression of oxygen production in HiRes compared to LoRes tanks, as would be expected due to the higher mean TBTO concentrations measured in LoRes versus HiRes tanks (1.2 versus 0.5 µg/L).



**Figure 10.** Integrated diurnal  $O_2$  production and consumption for HiRes and LoRes OMP-253 leachate treatment microcosms.



**Figure 11.** Integrated diurnal  $O_2$  production and consumption for HiOrg OMP-253 leachate treatment microcosms.

In marked contrast to these patterns, Pd climbed rapidly to levels that were 170 to 190 percent of control levels in the organic enrichment (HiOrg) tanks where TBTO concentrations were highest (figure 11). High Pd in those tanks correlated well with the abundance of green algae (*Cladophora socialis*), which was abundant through most of the treatment phase and decreased toward control levels during the recovery phase (section 2). A rich standing crop of *C. socialis* was apparently sustained largely by dissolved nutrient (nitrogen and phosphorus) subsidies provided by phytoplankton culture water. Over 30 percent of  $\text{P0}_4\text{-P}$  and  $\text{N0}_3\text{-N}$  added to HiOrg treatments was consumed by communities in those microcosms during the 1 hour of residence time of water in the tanks (section 1).

Moreover, as *Cladophora socialis* is apparently much more tolerant of organotins than most other species of algae, it would have had a considerable competitive advantage due to exclusion or reduction in abundance of other algae. Dissolved nutrient levels and phytoplankton abundances in most harbors are generally similar to those encountered in the HiOrg microcosms, and macroalgae in harbor environs are commonly dominated by chlorophytes such as *Cladophora*, *Ulva*, or *Enteromorpha*. Therefore, benthic organic productivity would be unaltered or possibly even enhanced by moderately high environmental concentrations of organotins.

Oxygen consumption rates in HiRes microcosms and LoRes microcosms remained at levels that were 63 to 87 percent of the control from the beginning through the end of treatment phase II (figure 10). Increased diurnal respiration or oxygen consumption (Rd) in HiRes tanks noted early in recovery phase II may have been caused by decomposition of declining populations of "organotin-acclimated" algae. By the end of recovery phase II, the Rd of HiRes and LoRes microcosms was near control levels.

Respiration in HiOrg microcosms climbed from below control levels at the beginning of treatment phase II to peaks of 127 and 134 percent of the control in the last quarter of the treatment period. Those peaks coincided with a maximum standing crop of algae in those microcosms and most likely represented plant respiration. Oxygen consumption rates of HiOrg communities were close to control levels at the termination of the experiment.

## General Discussion

Negative effects on the metabolism of benthic communities caused by leachates from equivalent paint areas ( $0.5 \text{ m}^2$ ) were minimal for F121 (copper-toxin paint) but were substantial for SPC-4 (organotin/cuprous thiocyanate-toxins paint). Strong suppression of oxygen production in SPC-4 tanks showed that plant communities were heavily impacted. Most of this impact was probably caused by the cuprous thiocyanate toxin as most soluble copper compounds are known to have algicidal properties. Cuprous thiocyanate may have higher relative toxicity than cuprous oxide (used in F121 paint) because it is not readily detoxified by chelation or adsorption as is the free copper ion that forms when cuprous oxide enters solution.

Negative effects of OMP-253 organotin were significant only in the low organic tanks that received fresh or unaged leachate. Oxygen production was actually higher than control levels in both the aged leachate and nutrient/organic enrichment tanks. As suggested by these results, the impact on organic productivity by sub- $\mu\text{g/L}$  organotin leachate concentrations at distances relatively far from point sources should be slight due to the combined effects of removal of organotins from water by degradation and adsorption/absorption processes, and the presence of pollution-adapted, high-productivity biota in many harbors.

All microcosms that received chronic leachate treatments exhibited near-normal metabolic rates after recovery periods of about 3 months. Thus, organic productivity of harbor benthic ecosystems would be expected to rapidly return to normal after being exposed for 2 to 3 months to TBTO concentrations of 0.5 to 2.0  $\mu\text{g/L}$ .

These metabolic data underscore the importance of monitoring stressed communities for relatively long periods of time to assess true pollutant impacts on ecosystems. Levels of oxygen production and respiration in most leachate-treated microcosms were seen to differ dramatically in early stages and at the termination of 3-month treatment phases. In most cases, relatively stable metabolic rates were attained only after 1 to 2 months of leachate exposure. These metabolic fluctuations reflected adjustments by the microcosms to leachate stress as tolerant organisms became established and functional relationships between species were altered. Most importantly, these changes required periods of weeks to months for natural recolonization and equilibrium of populations to occur. Therefore, short-term tests accomplished over periods of only a few hours to a few days would be expected to grossly misjudge the actual long-term impact of pollutants, such as antifouling leachates.

## SECTION 4.

# ORGANOTIN BURDENS OF MARINE ORGANISMS AND SEDIMENTS EXPOSED TO SPC-4 ANTIFOULING LEACHATES

## INTRODUCTION

The uptake and depuration rates of organotins by organisms and sediments are important factors in determining potential marine environmental impact of dissolved organotins. Also of interest, because of their differing levels of toxicity, are the chemical forms (species) of organotins present in biota and sediments. For example, trialkyltins are known to decrease in toxicity as they degrade and lose alkyl groups successively converting to di- and monoalkyltins (Maguire et al., 1983).

Measurements of organotin levels in organisms and sediments have been difficult to obtain because of several analytical problems. Some analysis techniques are subject to interferences from presence of sulfides and hydrocarbons, and most conventional digestion methods for separation of analytes cannot be used as the digestion processes alter organotin compounds so original organotin speciation is destroyed. On the other hand, nondestructive solvent extraction methods often recover low and variable amounts of total organotin present.

Development and optimization of extraction and analysis techniques for measurements of organotin in tissues and sediments are major elements in the NOSC program to evaluate environmental effects of antifouling paint leachates. In developing and testing those techniques, several analytical series were performed on samples collected from flowthrough microcosms that received chronic treatment of organotin leachates, followed by depuration or recovery periods. The number of analyses were relatively small, and uncertainties in the efficiencies and reproducibilities of the analytical technique were noted. However, several consistent patterns of uptake and release of organotins were seen in these preliminary data, and those results are presented in this report.

## METHODS

Organism and sediments samples were obtained from soft-bottom flowthrough microcosms that were subjected to a 93-day exposure of 0.8  $\mu\text{g/L}$  organotin-Sn leachate from SPC-4 (International Paint Company) antifouling paint. Samples were also collected from treated microcosms after 104 days of recovery (depuration) and from untreated (control) microcosms. Details of the experiment are available in other sections of this report.

*Bathygobius fuscus* (gobiid fish) and *Holothuria edulis* (sea cucumbers) were frozen immediately in polyethylene bags after collection from microcosms. Calcareous mud sediment samples of about 30-ml volumes were scooped from the upper 3 cm of sediment surfaces and were frozen immediately in polyethylene bags.

Each fish and sea cucumber sample was first ground in a tissue grinder and dried. One gram of homogenized sample was placed in an extraction thimble and was stoppered with a filter paper wad. The thimble was placed in a Soxhlet apparatus filled to a specified level with dichloromethane ( $\text{CH}_2\text{Cl}_2$ ). The flask was then heated to boiling for 16 hours of extraction after which the thimble was evaporated to dryness. The resultant dry sample was redissolved in 5 ml of methylisobutylketone (MIBK), transferred to a sample container, and analyzed for tin content on a graphite furnace atomic absorption spectrophotometer.

The residue of each fish sample that had been  $\text{CH}_2\text{Cl}_2$ -extracted was dried, weighed, and put into a round-bottom flask with 10 ml of Ultrex nitric acid per 1 g of sample. After 16 hours of boiling digestion, the sample was transferred to a volumetric flask, brought to a 50-ml volume with distilled water, and analyzed for tin on an atomic absorption spectrophotometer. This technique measured the amount of tin in the fish sample not recovered by  $\text{CH}_2\text{Cl}_2$  extraction. The sum of tin obtained by the two methods, therefore, represented both organic- and nonorganic-associated tin.

Sediment samples were air-dried and sifted through 350- $\mu\text{m}$  polypropylene mesh. From each sample, a weighed aliquot was put into a screw-capped test tube with about 1 ml of deionized water and 3 ml of MIBK. The tubes with contents were agitated on a rotary shaker for 24 hours, and the solvent was removed for analysis on an atomic absorption spectrophotometer.

Four sediment samples were analyzed for speciation of butyltins using a hydride reduction technique. For each analysis, about 300 mg of air-dried sediment was mixed with 500 ml of filtered seawater in a reaction chamber and was adjusted to pH 5 with acetic acid. Five millimoles of sodium borohydride were then added to the chamber, and the volatile tin hydrides formed were purged and trapped in liquid nitrogen. Tin compounds were then separated by differential boiling points and were measured by hydrogen flame atomic absorption spectrophotometry. Further details on the analysis of volatile tin hydrides are given in Valkers et al. (1985).

## RESULTS AND DISCUSSION

Total tin body burdens of gobiid fish exposed to 0.8  $\mu\text{g/L}$  of organotin-Sn leachate for 87 days had increased to levels about five times higher than control levels (table 6). After 104 days of recovery (depuration), tin body burdens of treated fish returned to control levels.

If total Sn accumulated above control levels by treated fish is assumed to be organotin-associated, gobiid organotin body burdens increased nearly 2,900 times above the mean water concentration in which the fish were maintained. This calculation also assumes that fish dry weight equals approximately 20 percent of wet weight. If organotin is present only in the  $\text{CH}_2\text{Cl}_2$ -extracted fraction, the accumulation factor would be about 1,300. In spite of the relatively high uptake of Sn, no direct negative impact of leachate was evident on the fish.



**Table 6.** Tin content of a gobiid fish (*Bathygobius fuscus*) maintained in microcosms for the F121/SPC-4 leachate effects experiment.

(as  $\mu\text{g Sn/g dry tissue}$ )

	Sn CH <sub>2</sub> Cl <sub>2</sub> - Extracted	Percent of Total	HNO <sub>3</sub> Digest of Residue	Percent of Total	Sn Total
Control	0.40 ( $\pm 0.22$ )	15	2.46 ( $\pm 0.95$ )	85	2.86 ( $\pm 1.15$ )
87-Day Exposure	5.46 ( $\pm 3.60$ )	38	8.91 ( $\pm 2.65$ )	62	14.37 ( $\pm 6.24$ )
104-Day Depuration	0.61 ( $\pm 0.36$ )	19	2.58 ( $\pm 1.63$ )	81	3.18 ( $\pm 1.99$ )

( $\pm$  SD for 3 values)

Another species of fish (*Cyprinodon variegatus*) maintained for 58 days under flowthrough conditions and a TBTO concentration of about  $1.6 \mu\text{g/L}$  ( $= 0.6 \mu\text{g Sn/L}$ ) showed tissue-to-water concentration ratios of 4,578 for viscera and 1,807 for muscle (Ward et al., 1981).

Percentages of total tin extracted by CH<sub>2</sub>Cl<sub>2</sub> and considered to be organically bound, or bound by weak chemical bonds, were less than 20 percent in control and depurated fish (table 6). As was expected, the percentage of CH<sub>2</sub>Cl<sub>2</sub>-extractable Sn was nearly twice as high in fish sampled immediately after the organotin exposure phase. However, a large increase in the total amount of acid-digestible Sn was also noted in exposure period fish, suggesting that a substantial amount of organotin assimilated by those fish may have been converted to nonorganic or tightly bound forms.

Another possibility, which should be examined in future tissue analysis, is that much of the Sn recovered by acid digestion may actually be organically or weakly bound tin present in tissue cells that may not have been ruptured during tissue grinding and, therefore, was not readily available for CH<sub>2</sub>Cl<sub>2</sub> extraction. More work should be done on determining the actual species of organotins present in fish tissues to evaluate potential toxicity to higher-order predators that feed on small fish.

Sea cucumbers exposed to  $0.8 \mu\text{g organotin-Sn/L}$  of SPC-4 leachate for 77 days were found to contain a mean body burden of  $8.0 \mu\text{g/g}$  ( $\pm 6.0$  SD, 3 analyses) dry tissue of CH<sub>2</sub>Cl<sub>2</sub>-extracted SN as compared to  $0.7 \mu\text{g/g}$  ( $\pm 0.1$  SD, 3 analyses) for controls. As CH<sub>2</sub>Cl<sub>2</sub> extraction recovered only about 38 percent of total organotin present in treated gobiid fish, total Sn body burdens were likely considerably higher than  $8.0 \mu\text{g/g}$  in the treated sea cucumbers. A high rate of bioaccumulation for sea cucumbers via processes other than ingestion of food is also implied. All treated individuals stopped feeding immediately when exposed to leachates and, when sampled, were found to have empty guts. In comparison, control individuals always fed normally in the microcosms and were found to have full guts when sampled.

Results of the analyses of mud sediments from the SPC-4 microcosm experiment revealed steady uptake of Sn throughout the 87-day treatment period, followed by rapid release of about 50 percent of the accumulated Sn burden through about 40 days of depuration (figure 12). Organotin depuration apparently continued at a slower rate after 40 days of recovery, indicating some organotin compounds may be more tightly bound to the sediments than the fractions lost during early depuration.

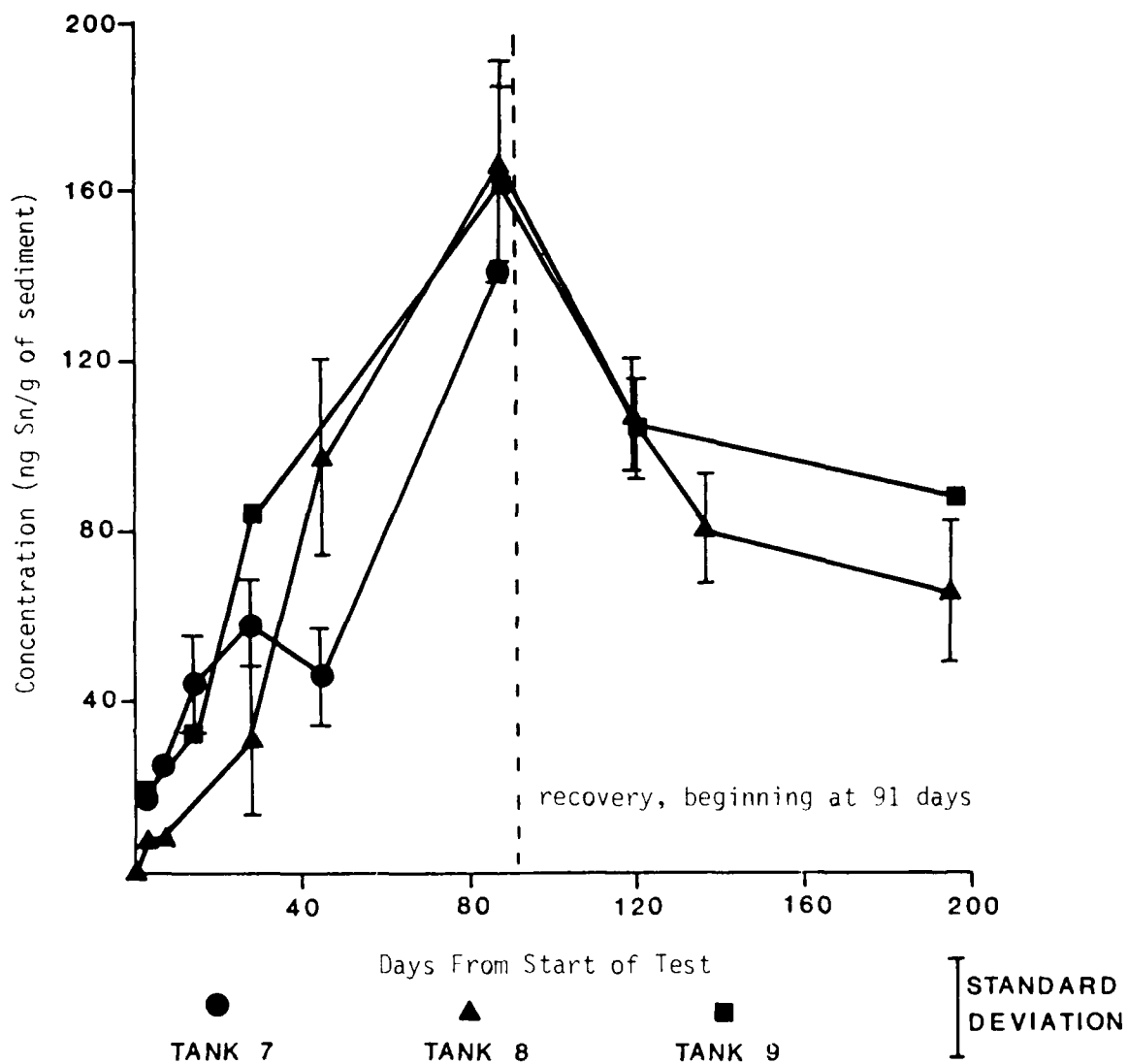
Considerable degradation of tributyltin in the microcosm sediments is indicated by data presented in table 7, with di- and monobutyltin comprising a mean of 30 percent and 32 percent, respectively, of total organotin values. Although microbial or chemical processes may be responsible for this degradation, photodecomposition may also be involved because the water depth over microcosm sediments was less than 0.5 m.

Total solvent extractable Sn values measured in the 87-day SPC-4-exposed microcosm sediments and sediments collected from harbor bottoms at Matson Pier in Honolulu Harbor and the Commercial Basin in San Diego Harbor (Grovehoug et al., in prep.) fall within a narrow range of 141 to 164 ng Sn/g sediment. Both harbor sites contain substantial numbers of craft painted with organotin paints. Control sediments from microcosm and field sites contained only 10 to 20 ng Sn/g sediment. The similar sediment levels seen in the microcosm and field sites, which received moderate to high dissolved organotin loading, suggest the possibility of a mechanism that controls or limits the total amount of organotins accumulated by fine-grained sediments. Data to be obtained in upcoming microcosm experiments and field measurements will be examined closely for evidence of such a limit on organotin uptake.

**Table 7.** Concentration of total organotin and organotin species in microcosm sediments exposed to SPC-4 leachates.

Days Exposure	Total HFAAS Organotin	<u>Bu<sub>3</sub>SnCl</u>		<u>Bu<sub>2</sub>SnCl<sub>2</sub></u>		<u>BuSnCl<sub>3</sub></u>	
	μg/kg	μg/kg	Percent of total	μg/kg	Percent of total	μg/kg	Percent of total
14	284	101	35.5	106	37.0	77	27.0
45	339	132	38.9	94	27.7	113	33.3
87	480	181	37.7	123	25.6	176	36.7
Control	< 30	< 10		< 10		< 10	

HFAAS = Hydrogen flame atomic absorption spectrophotometry.  
Speciated analysis performed by hydride reduction technique.



**Figure 12.** Uptake and depuration of organotin-Sn in microcosm sediments exposed to a mean concentration of 0.8  $\mu\text{g/L}$  of organotin-Sn SPC-4 leachate.

## SECTION 5.

### EFFECTS OF ANTIFOULING LEACHATES ON TWO SPECIES OF CORAL

#### INTRODUCTION

Many species of inshore stony corals are potentially useful as bioindicators of various types of environmental stress. Because of their relatively long life spans of at least several years and their sedentary habits, colonies of most coral species serve as good temporal integrators of environmental conditions. Most inshore corals can be maintained in flowthrough research facilities and are easily observed while revealing a broad spectrum of visible responses to perturbants. Stress responses are manifested in symptoms including excess mucus production, loss of zooxanthellae pigment, retraction of tentacles, and polyp mortality.

This section reports the responses of two common Indo-Pacific corals, *Pocillopora damicornis* and *Montipora verrucosa*, to low-level ( $\mu\text{g/L}$ ) concentrations of dissolved copper and organotin compounds leached from antifouling paints. These coral species were selected for use primarily because they have widespread geographic distribution, are readily available in small colony size, and are relatively hardy in captivity. Additionally, they have been the focus of a variety of studies that have measured their sensitivity to factors such as temperature (Coles & Jokiel, 1978; Jokiel & Guinther, 1978), light (Franzisket, 1970; Jokiel & York, 1982), water motion (Jokiel, 1978), and dissolved copper (Evans, 1977). The inclusion of colonies of the two coral species in the present series of experiments allowed precise estimations of subtle toxicity differences between treatments to be made.

#### METHODS

In these experiments, corals were exposed to antifouling leachate in 465-L flowthrough microcosms harboring soft-bottom communities. During the treatment phase of the first experiment, corals were placed into two control tanks (no leachates), two Navy F121 paint leachate tanks (with a mean concentration of cuprous oxide-derived Cu of  $5.6 \mu\text{g/L}$ ), and two International Paint Company SPC-4 paint leachate tanks (with a mean concentration of cuprous thiocyanate-derived Cu of  $1.3 \mu\text{g/L}$  and mean organotin-Sn concentration of  $0.8 \mu\text{g/L}$ ).

Colonies of the two species of coral (*Pocillopora damicornis* and *Montipora verrucosa*) were collected in Kaneohe Bay, from depths of 1 to 3 m, and were allowed 1 week of adaptation in flowthrough holding tanks before introduction to the experimental tanks. On the 50th day of the leachate treatment phase, six healthy colonies of each coral species were put into each microcosm. Thereafter, the percentage of live polyp coverage, the degree of overall polyp distention (or retraction), and pigmentation were monitored every few days on all colonies. The corals were removed from the microcosm tanks at the end of the treatment phase.

Corals of the same species and collection locations as those of the first experiment were allowed 12 days of adaptation in flowthrough tanks and were used as study subjects in the second experiment. Seven colonies of each of the two coral species were added to one tank of each treatment on the 56th day of low-level leachate exposure (treatment phase II). Mean concentrations of TBTO in various treatments of OMP-253 antifouling paint leachates were 0.5  $\mu\text{g/L}$  for an aged leachate tank (HiRes), 1.2  $\mu\text{g/L}$  for an unaged leachate tank (LoRes), and 1.7  $\mu\text{g/L}$  for an unaged leachate tank that also received continuous input of phytoplankton to elevate particulate organic loading (HiOrg). Health and appearance of the corals were monitored as in the first experiment, and colonies were removed at the end of the treatment phase.

Further details on the chemistry and biology of the two leachate-effects microcosm experiments are given in the other sections of this report.

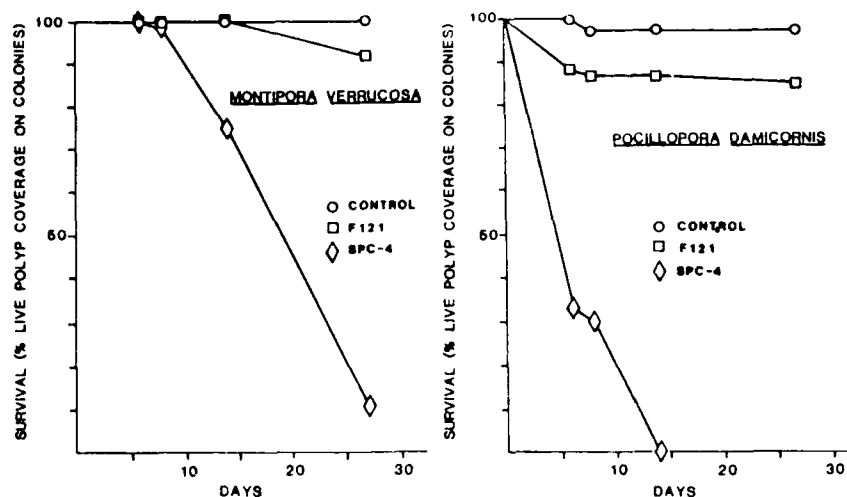
## RESULTS AND DISCUSSION

Within hours of being placed into SPC-4 leachate tanks, both species of corals (*P. damicornis* and *M. verrucosa*) began to secrete copious amounts of mucus. One week later, 8 of 12 colonies of *P. damicornis* in SPC-4 microcosms were dead (figure 13), and 5 to 90 percent of tissues on the remaining colonies lacked pigmentation. Most pigment in these two species of coral is derived from algal symbionts living within the coral tissues. Therefore, loss of pigmentation in these corals is indicative of loss of pigments in the zooxanthellae or expulsion of zooxanthellae by the host coral tissues.

Most tissues on *Montipora verrucosa* colonies were alive after 1 week of SPC-4 exposure, but all polyps on those colonies were retracted and were covered with patchy layers of mucus, diatoms, and detritus. After 2 weeks of SPC-4 exposure, all *Pocillopora damicornis* colonies were dead, and at 4 weeks only 10 percent of *M. verrucosa* polyps were alive (figure 13).

Survival of corals in the F121 leachate tanks was only slightly below control survival (figure 13), but the two coral species showed differing sublethal responses to copper enrichment. After 4 weeks of exposure, all live *P. damicornis* polyps were normally distended and pigmented. In comparison, approximately half of the *M. verrucosa* colonies remaining after 4 weeks of exposure were lightly pigmented with polyps retracted and covered with mucus. Other live *M. verrucosa* colonies were normal.

The same two coral species suffered 100-percent mortality within 6 days in a previous microcosm experiment (Evans, 1977) under exposure to 8.7  $\mu\text{g/L}$  of  $\text{CuSO}_4$ . Thus, threshold copper concentrations for long-term survivability of these corals are probably between 5.6 and 8.7  $\mu\text{g/L}$  for *P. damicornis* and below 5.6  $\mu\text{g/L}$  for *M. verrucosa*. Notably, no species of hard corals are found in Pearl Harbor, where copper concentrations have been found to range from 2 to 14  $\mu\text{g/L}$  (R. S. Henderson, 1976 survey, unpublished data).



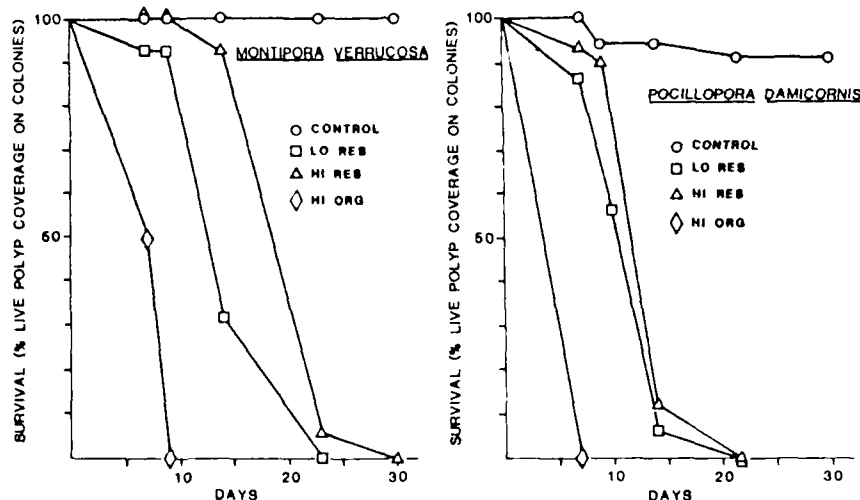
**Figure 13.** Survival of two coral species (*Montipora verrucosa* and *Pocillopora damicornis*) exposed to leachates from 0.1 m<sup>2</sup> of F121 (copper toxin) or SPC-4 (organotin and copper toxins) paint.

Both coral species exhibited retraction of polyps and loss of most pigmentation after 2 days of exposure to all OMP-253 leachate treatments. These stress responses were similar to those displayed by corals exposed to SPC-4 leachate in the first experiment. Mucus production by OMP-253-exposed corals, however, was low as compared to that of SPC-4 exposed corals. Cuprous thiocyanate present in SPC-4 (but not present in OMP-253) may possibly be the primary substance that elicited mucus output in corals exposed to that leachate.

By the 23rd day of OMP-253 exposure, both coral species had sustained 100-percent mortality in all treatments except for *M. verrucosa* colonies in the aged leachate (HiRes) tank, which showed only 6-percent live tissue remaining (figure 14). Toxicity to corals was clearly highest in the organically enriched leachate (HiOrg) treatment where 50 percent of *M. verrucosa* and 100 percent of *P. damicornis* polyps were killed in the first week of exposure. As noted earlier, mean total organotin concentrations were also highest in that treatment. Higher toxicity in the unaged leachate (LoRes) tanks as compared to the aged leachate (HiRes) tank also conformed to the hierarchy of organotin concentrations (and expected toxicities) measured in the microcosm tanks.

Toxic responses of *Montipora verrucosa* in the HiRes and LoRes tanks paralleled fairly closely the responses of the same coral species to SPC-4 leachate (figures 13 and 14). However, mortality of *Pocillopora damicornis* occurred at a more rapid rate in the SPC-4 tank than in LoRes and HiRes tanks of the OMP-253 treatments. This indicates *P. damicornis* may be much less tolerant to cuprous thiocyanate (or combined effects of cuprous thiocyanate and organotin) than *M. verrucosa*.

These experiments demonstrate that the two common inshore species studied are very sensitive indicators of  $\mu\text{g/L}$  concentrations of dissolved organotins. Even sub- $\mu\text{g/L}$  differences in total organotin concentrations were readily distinguishable by toxic responses of the corals. Those responses also typified the overall toxic impacts of leachates on the microcosm benthic communities in general. Coral bioassays could, therefore, probably be used to provide quantitative estimations of toxicities of various antifouling leachates on complex marine communities.



**Figure 14.** Survival of two coral species (*Montipora verrucosa* and *Pocillopora damicornis*) exposed to various treatments of OMP-253 paint leachates from a  $0.1 \text{ m}^2$  panel.

Over equivalent intervals, copper (F121) exposure generated only about one-tenth the mortality observed in organotin treatments. As copper concentration on a molar basis was nearly nine times higher than the highest tin concentration, the toxicity of organotin-Sn to corals was at least an order of magnitude higher than that of copper.

Effects of cuprous thiocyanate leachates were difficult to separate from those of the organotin leachate in the SPC-4 treatments. However, very high mucus production by SPC-4 exposed corals, which was not seen in OMP-253-exposed corals, suggests that cuprous thiocyanate generates at least some of the negative effects in these organisms. Experiments exposing corals to cuprous thiocyanate alone should be performed to delineate toxic effects of that leachate component.

The fact that both species of coral lose zooxanthellae pigment and retract polyps permanently very soon after being exposed to  $0.5 \mu\text{g/L}$  of TBTO implies that corals may lose their ability to feed and metabolize normally. Thus, they may suffer high mortality over longer periods of exposure to TBTO concentrations lower than  $0.5 \mu\text{g/L}$ .

## REFERENCES

- Chesher, R. H. (1975). "Biological impact of a large-scale desalinization plant at Key West, Florida." In: Tropical Marine Pollution. eds, E.J.F. Wood and R.E. Johannes. Elsevier Scientific Publishing Co., New York, pp. 99-153.
- Coles, S.L., and P.L. Jokiel (1978). "Synergistic effects of temperature, salinity and light on the hermatypic coral *Montipora verrucosa*." Mar. Biol. 49, pp. 187-195.
- Dooley, C., and V. Homer (1983). Organotin compounds in the marine environment: uptake and sorption behavior. (NOSC Tech. Rep. 917). San Diego: Naval Ocean Systems Center.
- Draggan, S., and J.J. Reisa (1980). "Controlling toxic substances: historical perspectives and future research needs." In: Microcosms in Ecological Research. ed. J.P. Giesy. U.S. Dept. of Energy Symp. Series 52 (CONF-781101), pp. iii-xii.
- Dundee, D.S., R. Giardina, G. Swindler, and M.L. Good (1980). "Biological effects of organotin antifoulants." In: Proc. 7th Int. Symp. on Releases of Bioactive Materials. Ft. Lauderdale, Florida, 28-30 July 1980, pp. 55-59.
- Evans, E.C. (1977). "Microcosm responses to environmental perturbants." Helgolander Wiss. Meeresunters 30, pp. 160-168.
- Evans, E.C., and D.E. Morris (1974). "Water quality." In: Pearl Harbor Biological Survey — Final Report. ed. E.C. Evans. Naval Undersea Center Tech. Note 1128, pp. 3.2/1-45
- Franzisket, L. (1970). "The atrophy of hermatypic reef corals maintained in darkness and their subsequent regeneration in light." Int. Revue ges Hydrobiol. 55(1), pp. 1-12.
- Giesy, J.P. (ed.) (1980). "Microcosms in Ecological Research." U.S. Tech. Info. Center Pub., U.S. Dept of Energy Symp. Series 52 (CONF-781101), 1110 pp.
- Grice, G.D., and M.R. Reeve (eds.) (1982). Marine Mesocosms. Springer-Verlag Inc., New York, 430 pp.
- Grovhough, J.G., P.F. Seligman, W.J. Cooke, and R.L. Fransham (in prep.). Baseline Inventory of Butyltins in Navy Harbors. Naval Ocean Systems Center Technical Report in preparation.
- Grovhough, J.G. (1979). Marine environmental assessment at three sites in Pearl Harbor, Oahu, August-October 1978. (NOSC Tech. Rep. 441). San Diego: Naval Ocean Systems Center.
- Grovhough, J.G., and E.B. Guinther (1974). "Benthic survey." In: Pearl Harbor Biological Survey — Final Report. ed. E.C. Evans. Naval Undersea Center Tech. Note 1128, pp. 2.2/1-60.



## REFERENCES (Continued)

- Henderson, R.S., and S.V. Smith (1980). "Semi-tropical marine microcosms: facility design and an elevated nutrient effects experiment." In: Microcosms in Ecological Research, ed. J.P. Giesy. U.S. Dept. of Energy Symp. Series 52, (CONF-781101), pp. 869-910.
- Homer, V., and C. Dooley (1983). Quantification of tin and organotin in seawater, sediments, and biological materials by graphite furnace atomic absorption spectroscopy. (NOSC Tech. Rep. 918). San Diego: Naval Ocean Systems Center.
- Jokiel, P.L. (1978). "Effects of water motion on reef corals." Jour. Exp. Mar. Biol. Ecol. 35, pp. 87-97.
- Jokiel, P.L., and E.B. Guinther (1978). "Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*." Bull. Mar. Sci. 28(4), pp. 786-789.
- Jokiel, P.L., and R.H. York (1982). "Solar ultraviolet photobiology of the reef coral *Pocillopora damicornis* and symbiotic zooxanthellae." Bull. Mar. Sci. 32(1), pp. 301-315.
- Kenis, P., and A. Zirino (1983). "Quantitative measurement of tributyltin oxide in sea water by differential pulse anodic stripping voltammetry." Analytica Chimica Acta 149, pp. 157-166.
- Maquire, R.J., J. H. Carey, and E.J. Hale (1983). "Degradation of the Tri-n-butyltin Species in Water." Jour. Agric. Food Chem., 31(5), pp. 1060-1065.
- Smith, S.V., P.L. Jokiel, G.S. Key, and E.B. Guinther (1979). Metabolic responses of shallow tropical benthic microcosms to perturbation. U.S. Environ. Protection Agency Report EPA-600/3-79-061, 57 pp.
- Smith, S.V., W.J. Kimmerer, E.A. Laws, R.E. Brock, and T.W. Walsh (1981). "Kaneohe Bay sewage diversion experiment: perspectives on ecosystem responses to nutritional perturbation." Pac. Sci. 35, pp. 295-395.
- Strickland, J.D.H., and T.R. Parsons (1972). A Practical Handbook of Seawater Analysis. Bull. 167. Fisheries Res. Bd. of Canada, Ottawa, 310 pp.
- Valkirs, A.O., P.F. Seligman, G. Vafa, V. Homer, P. Stang, and S.H. Lieberman (1985). Speciation of butyltins and methyltin in seawater and marine sediments of hydride derivatization and atomic absorption detection. (NOSC Tech. Rep. 1037). San Diego: Naval Ocean Systems Center.
- Ward, G.S., G.C. Cramm, P.R. Parrish, H. Trachman, and A. Slesinger (1981). "Bioaccumulation and chronic toxicity of bis(tributyltin) oxide (TBTO): test with a saltwater fish." In: Aquatic Toxicology and Hazard Assessment: 4th Conference, ASTM STP-737, eds. D.R. Branson and K.L. Dickson, Amer. Soc. For Testing and Materials, pp. 183-200.

## ABBREVIATIONS AND SYMBOLS

CH <sub>2</sub> Cl <sub>2</sub>	Dichloromethane
cm	Centimeter
Contr	Control microcosm in OMP-253 experiment. One-hour residence time of water in microcosm tank and no leachate present.
CuSO <sub>4</sub>	Copper sulfate
°C	Degrees Celsius
DBT	Dibutyltin
DOC	Dissolved organic carbon
F121	Navy Mil Spec copper-based antifouling paint F121/63 formulation
F150	Navy Mil Spec epoxy primer paint
g	Gram
GF/C	Glass fiber filter/coarse grade
HiOrg	OMP-253 microcosm treatment enriched with phytoplankton culture. One-hour residence time of water and leachate in tank.
HiRes	OMP-253 microcosm treatment receiving aged (5.7 hours) OMP-253 leachate and water from HiRes-Pool. Seawater residence time in HiRes tank was 1 hour.
HiRes-Pool	2400-L pool that provided aged water and leachate to OMP-253 HiRes tank treatments. Seawater residence time in pool was 5.7 hours.
kg	Kilogram
L	Liter
LoRes	OMP-253 microcosm treatment receiving low-organic seawater. One-hour residence time of water and leachate in microcosm tank.
m	Meter
MBT	Monobutyltin
mg	Milligram
MIBK	Methylisobutylketone
min	Minute
ml	Milliliter
mm	Millimeter

## ABBREVIATIONS AND SYMBOLS (Continued)

$\mu\text{g}$	Microgram
$\mu\text{m}$	Micrometer
N	Nitrogen
ND	No detection
ng	Nanogram
$\text{NH}_4\text{-N}$	Ammonium nitrogen
$\text{NO}_3\text{-N}$	Nitrate nitrogen
$\text{O}_2$	Oxygen
OMP-253	Organometallic polymer antifouling paints #253 formulation (developed by David W. Taylor Naval Ship Research and Development Center)
P	Phosphorus
Pd	Diurnal (24 hour) oxygen production
PIM	Particulate inorganic material
$\text{PO}_4\text{-P}$	Phosphate phosphorus
POC	Particulate organic carbon
Pp	Peak oxygen production
ppb	Parts per billion ( $\mu\text{g/liter}$ )
ppt	Parts per thousand
Rd	Diurnal (24 hour) respiration or oxygen consumption
Rp	Peak respiration or oxygen consumption
SD	Standard deviation
Sn	Tin
sp.	species
SPC-4	Self-polishing copolymer antifouling paint (developed by International Paint Company)
TBTO	Bis(tri-n-butyltin) oxide
TBT	Tributyltin cation
V	Number of values or data points